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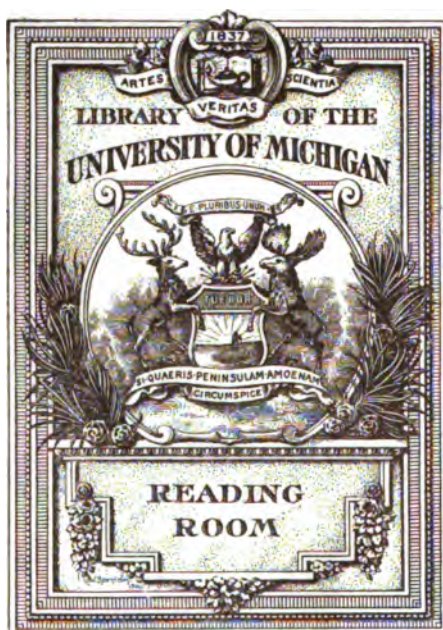
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PRACTICAL SANITARY SCIENCE



PRACTICAL SANITARY SCIENCE

A HANDBOOK FOR THE PUBLIC
HEALTH LABORATORY

BY

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OF ST. THOMAS'S HOSPITAL

SECOND EDITION

NEW YORK
WILLIAM WOOD & COMPANY
MDCCCCXV

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201

PREFACE
TO
THE SECOND EDITION

THE arrangement in chapters has been altered, and considerable additional matter has been added.

The introduction to qualitative chemical analysis has been discarded in order to prevent increase in size of the book.

Some less frequently occurring operations are outlined in a brief appendix.

D. S.

UNIVERSITY OF LONDON KING'S COLLEGE,
November, 1914.

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PREFACE TO THE FIRST EDITION

THIS little book is a brief summary of the course of practical lecture-demonstrations given to the D.P.H. class at King's College, London. Its intention is to put in the hands of students working in laboratories of Public Health a short outline of the more important matters—chemical, physical, etc.—discussed at practical examinations in Sanitary Science.

The methods described are few, but it is hoped they will be found reliable. It is felt that where a large field must be cultivated in a limited time, it is better to use a few tools which have been well tried. Whilst going through the work, the student will do well to constantly refer to elementary up-to-date textbooks in the subjects of experimental physics, systematic organic and inorganic chemistry, analytical chemistry, geology, and bacteriology. Further, it will be necessary for him at the outset to bear in mind that no amount of theoretical reading can be made a substitute for the laborious and constant use of the test-tube, microscope, etc., which must take place at the benches.

A short account of the preparation of the standard solutions referred to in the work, and a few brief notes on the general chemical reactions of the more commonly occurring metals and acids, are set out in an appendix.

D. S.

KING'S COLLEGE,
November, 1905.

CONTENTS

| CHAPTER | PAGE |
|---|------|
| I. GENERAL OBSERVATIONS UPON POTABLE WATERS IN RELATION TO THEIR SOURCE, AND METHODS OF EXAMINATION ADOPTED FOR SAFEGUARDING THEIR PURITY - - | 1 |
| II. THE PHYSICAL EXAMINATION OF WATER - - - | 7 |
| III. THE CHEMICAL EXAMINATION OF WATER - - - | 12 |
| IV. ORGANIC MATTER IN WATER - - - | 38 |
| V. OXIDIZED NITROGEN—NITRITES AND NITRATES - - | 51 |
| VI. GASES IN WATER—WATER SEDIMENT—INTERPRETATION OF RESULTS OF CHEMICAL ANALYSES - - - | 60 |
| VII. THE BACTERIOLOGY OF WATER—EXAMPLES OF WATERS FROM VARIOUS SOURCES - - - - - | 83 |
| VIII. SEWAGE EFFLUENTS - - - - - | 97 |
| IX. SOIL - - - - - | 105 |
| X. AIR - - - - - | 118 |
| XI. FOODSTUFFS: MILK—BUTTER—CHEESE—CEREALS—BREAD—MEAT—ALCOHOLIC BEVERAGES—LIME AND LEMON JUICES—VINEGAR—MUSTARD—PEPPER—SUGAR—TEA—COFFEE—COCOA - - - - - | 149 |
| XII. DISINFECTANTS - - - - - | 287 |
| APPENDIX - - - - - | 313 |
| INDEX - - - - - | 321 |

LIST OF ILLUSTRATIONS

| FIG. | PAGE | FIG. | PAGE |
|------------------------------------|------|---|------|
| 1. Geological fault, etc. - | 4 | 49. <i>Tilletia caries</i> (<i>Uredo fo-</i> | |
| 2. Curve of ground-water - | 5 | <i>tida</i>) - - - - | 224 |
| 3. Diagrammatic scheme of | | 50. Wheat-stem infected with | |
| organic pollution under- | | <i>puccinia</i> - - - | 225 |
| going purification - | 40 | 51. Portion of Fig. 50 more | |
| 4. Thresh's apparatus - | 61 | highly magnified - - | 226 |
| 5-22. Objects found in water | | 52. Teleutospores - - - | 226 |
| sediments - 69, 70, 71, 72, | | 53. <i>Æcidium berberidis</i> - | 226 |
| 73, 74, 75, 76, 77, 78 | | 54. Gonidiospores and teleuto- | |
| 23. Adeney's apparatus - | 103 | spore - - - - | 226 |
| 24. Barometer and vernier | | 55. Ergot in rye - - - | 227 |
| scales - - - - | 121 | 56. <i>Sclerotium</i> - bearing stro- | |
| 25. Hempel's gas burette and | | mata - - - - | 228 |
| absorption pipette - | 131 | 57. Stroma containing asco- | |
| 26. Apparatus used in milk | | carps - - - - | 228 |
| analysis - - - - | 158 | 58. Ascocarp containing asci - | 228 |
| 27. Apparatus used in milk | | 59. Ascus containing ascospores | 228 |
| analysis - - - - | 160 | 60. Head of <i>cysticercus</i> - | 240 |
| 28. Apparatus used in butter | | 61. <i>Tænia solium</i> - - - | 240 |
| analysis - - - - | 186 | 62. <i>Trichina spiralis</i> - | 241 |
| 29. Granules of wheat starch - | 215 | 63. Head of <i>Distoma hepaticum</i> | 241 |
| 30. Granules of barley - - - | 215 | 64. <i>Ascarus lumbricoides</i> - | 242 |
| 31. Granules of rye - - - | 216 | 65. <i>Oxyuris vermicularis</i> - | 243 |
| 32. Granules of rice - - - | 216 | 66. Apparatus used in estima- | |
| 33. Granules of oat - - - | 217 | tion of alcohol - - - | 252 |
| 34. Granules of maize - - - | 217 | 67. Cells of cuticle of mustard - | 269 |
| 35. Granules of sago - - - | 218 | 68. Black pepper - - - | 270 |
| 36. Granules of tapioca - - - | 218 | 69. Cuticle of tea-leaf - - - | 276 |
| 37. Granules of pea - - - | 219 | 70. Idioblasts in section of tea- | |
| 38. Granules of haricot bean - | 219 | leaf - - - - | 276 |
| 39. Granules of arrowroot - - | 220 | 71. Tea-leaf - - - - | 276 |
| 40. Granules of potato - - - | 220 | 72. Elder-leaf - - - - | 278 |
| 41. <i>Vibrio tritici</i> - - - | 221 | 73. Willow-leaf - - - - | 278 |
| 42. <i>Bruchus pisi</i> - - - | 221 | 74. Sloe-leaf - - - - | 278 |
| 43. <i>Acarus farinae</i> - - - | 221 | 75. Cuticle of tobacco-leaf - | 279 |
| 44. <i>Penicillium glaucum</i> - - | 222 | 76. Coffee-berry - - - | 282 |
| 45. <i>Aspergillus glaucus</i> - - | 222 | 77. Ground coffee, showing cells | |
| 46. <i>Mucor mucedo</i> - - - | 222 | of testa - - - - | 283 |
| 47. <i>Peronospora</i> - - - | 222 | 78. Lacteal vessels of chicory - | 284 |
| 48. <i>Ustilago segetum</i> - - - | 223 | 79. Dotted vessels of chicory - | 284 |

PRACTICAL SANITARY SCIENCE

CHAPTER I

GENERAL OBSERVATIONS UPON POTABLE WATERS IN RELATION TO THEIR SOURCE, AND METHODS OF EXAMINATION ADOPTED FOR SAFEGUARDING THEIR PURITY

WATER is often the vehicle of infectious diseases, poisonous metallic salts, and a large number of undesirable materials—animal, vegetable, and mineral. When we consider how drinking waters are obtained, and how liable they are to contamination at all points from source to final distribution, it will be readily admitted that every potable water should be the object of the most careful, intelligent, and constant concern. The primary object of a water analysis for public health purposes is to ascertain whether or not it contains sewage, as in the organic matter contributed by sewage are found the organisms of infectious disease, such as *Bacillus typhosus*, *Vibrio cholerae asiaticæ*, etc. All other information is of very secondary import compared with this. The detection of organic filth, whether of animal or vegetable origin, and of harmful inorganic matters, when in small quantities, is often a work of no little difficulty. In certain cases where a small amount of sewage containing pathogenic micro-organisms finds its way into a water-supply, no chemical analysis, however delicate, can furnish evidence of the pollution. So also in other cases the most exact bacteriological examination may wholly fail to discover a dangerous water. The well-informed analyst will not pin his faith to one method of examination to the partial or total exclusion of others, but will welcome all reliable methods that can assist in throwing light on his search.

At present four methods of examination are utilized—viz., Physical, Chemical, Biological, Bacteriological—each of which has its place and its limits.

The physical examination may detect pollution so gross that further inquiry is unnecessary.

The chemical analysis can render no information concerning liability to contamination, and is useless in detecting small quantities of sewage. A systematic chemical analysis is of value in demonstrating variations in character produced, for example, by the lowering of the level of well waters, by change in rainfall, action on lead, iron, and zinc, in pipes, mains, cisterns, boilers, etc. Where the estimation of saline constituents must be determined for health purposes, manufacturing and engineering purposes, etc., the chemical method alone is of value. Here it may be stated as a general principle that waters most suitable for domestic purposes are also most suitable for manufacturing and engineering purposes. Acid waters corrode boilers, so do waters containing marked quantities of $MgCl_2$ and $CaCl_2$, as these chlorides at high temperatures decompose, forming HCl , which at once attacks the iron. $CaSO_4$, being insoluble, is deposited as a crust. $CaCO_3$ and $MgCO_3$ together with salts of Fe render water unsuitable for tanning, dyeing, paper-making, and other industries, owing to their great insolubility, whereby particles are left in the fabrics.

Neutral and alkaline (Na_2CO_3) waters are best suited for boilers.

Special chemical analyses are required in dealing with medicinal waters.

By careful and systematic study of the lower forms of animal and vegetable life, much information may be acquired as to the source and mode of entry of surface waters into water-supplies. Such biological examination has not had in this country the attention it deserves.

Where the question of infective micro-organisms in water arises, which to the sanitarian is of all questions the most important, the bacteriological examination only can afford positive evidence.

The examination of the source of a water-supply is of the first import, and should never be omitted. Personal inspection of the catchment area, all streams arising therefrom, and all feeders of such streams, should be made *in situ*, and the relations of these

to possible sources of pollution carefully noted. When the gathering ground has been thoroughly investigated, attention should be turned to the storage reservoirs, and finally the efficiency of filtration should be bacteriologically tested. Such examination presupposes an intimate knowledge of the entire area set apart for collection, which should be protected from all possibility of contamination from manured soil, house drainage, and storm waters. A good working knowledge of the geology of the district is essential, and every student of water analysis should intimately cultivate the solid and drift maps of the Ordnance Survey.

The following brief table gives an outline of the more important strata in this country, detailed descriptions of which will be found in any textbook of geology.

Post-tertiary deposits:

Alluvium, sands, gravels, boulder clay.

Tertiary deposits:

Sands of the Eastern English counties.

Bagshot sands (upper, middle, and lower).

London clay.

Secondary deposits:

Chalk.

Greensands—upper and lower—with gault lying between.

Weald clay.

Purbeck marble.

Kimmeridge clay.

Oolite.

Lias.

New red sandstones.

Primary deposits:

Coal, ironstones.

Limestone.

Old red sandstones.

Shales and slates.

Crystalline rocks.

Shallow wells sunk in the post-tertiary sands and gravels are very liable to pollution.

The Bagshot sands yield a fairly soft water.

The London clay is an impervious stratum, and the waters resting immediately on it are generally hard.

The chalk formations of England, which are extensive, yield both hard and soft waters. The hardness is mostly temporary. Fissures make it possible for pollution to readily get access to these waters.

The greensands, especially the lower, bear waters rich in calcium and iron salts.

Oolites produce waters almost identical with those of the chalk.

The magnesium limestones (dolomite) and new red sandstones give origin to much hardness, of which a large portion is permanent.

Slates and igneous rocks, being practically insoluble, yield waters destitute of saline matters, and are consequently very soft.

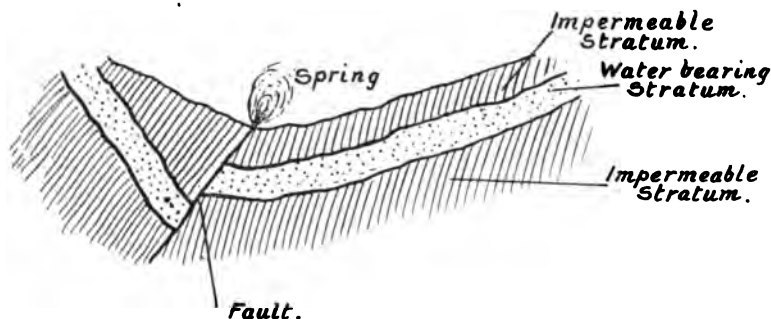


FIG. 1.

The drainage area of a well depends upon the depth of the well, the porosity of the soil and subsoil, direction of flow of ground water, and the daily depression produced by pumping. It may be considered as the base of a cone whose apex is the water-level in the well.

Even with a good knowledge of the geology of the catchment area and districts through which the water passes, the analyst is subject to pitfalls at all points. Strata may contain caverns and fissures which lodge pollution in the most unlikely positions. Geological faults account for unexpected positions of springs. Where a water-bearing, permeable stratum intervenes between two impermeable strata, and a fault occurs, the imprisoned fluid may become subject to such pressure that it escapes at the surface with tremendous force.

It is to be noted that the curve of the ground water near the well is steep, but rapidly shades off into the horizontal. It is obvious that with different types of soil the form of this curve changes as the surface water in the well is lowered. The drainage area increases in direct proportion to the porosity.

This area should be protected from all forms of organic pollution, including cultivated soils, and it has been laid down as a minimum requirement that it should have a radius of twenty times the maximum depression of the water through pumping—*e.g.*, if the depression in the well be 5 feet, the area should have a radius of 100 feet, etc. Outside this cone it is considered that filtration is so slow that purification is complete. A wide margin, however,

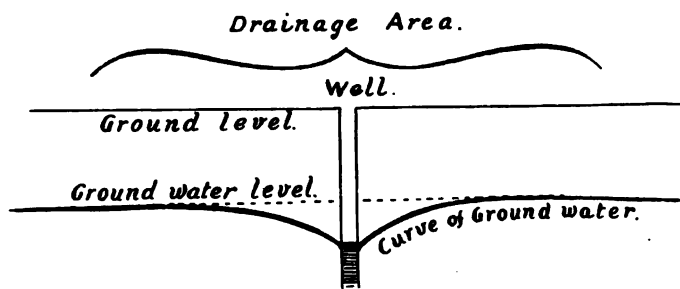


FIG. 2.

should be allowed in the drainage area to meet the effects of increased rainfall, possible faults in the brickwork of the well, and other factors, so that wells supplying drinking waters should be removed widely from all sources of drainage, farmyard manure, etc. A slight acquaintance with the situations of many rural wells in this country must call forth unqualified condemnation. There is no doubt that many epidemics of typhoid fever have their origin in the waters of these wells. It is a matter of little difficulty to determine whether or not leakage from the immediate surroundings takes place into a well, and an alkaline solution of fluorescein, an emulsion of *Bacillus prodigiosus*, or a concentrated solution of NaCl, poured around its mouth and thoroughly washed into the soil, will afford the necessary evidence within a limited time.

Peat which lies for the most part on igneous rocks imparts to

water certain organic acids capable of dissolving metals. Wherever possible such waters should be cut out of a supply. If this cannot be done the acids should be neutralized before the waters pass to the consumer.

Rivers and streams from which water-supplies are procured should be scrupulously preserved from the entrance of pollution, with a special view to the exclusion of infective bacteria. All river water should be sedimented and filtered before use, and the efficiency of filtration should be constantly tested by bacteriological examination.

All forms of animal and vegetable life should be excluded from service reservoirs, cisterns, mains, etc. It is well known that certain low vegetable forms, especially when dead, give origin to offensive odours.

Water moves in a cycle. Evaporation produces clouds, which return to the earth as rain. This rain, according to the nature of the soil, subsoil, and rocks, pursues various paths. If it fall on impervious granite it runs off in large quantity; a part may be evaporated, and this will occur to the greatest degree during dry, hot, and windy weather. If it fall on sandy soil a large proportion percolates, and the more porous and deep the sand, the more rapidly and deeply the water sinks into the earth. When it meets with an impermeable stratum its further course is directed by the slope and contour of this stratum. Should the latter take the form of a basin the water will accumulate until it overflows the lip of the basin, forming a spring at a point where the stratum outcrops. Again, if the stratum form an inclined plane, as on the sides of a river valley, the water will flow along the plane to its outlet at the lowest point. Such pure waters may be intercepted before reaching polluted rivers by sinking wells at the bases of the hills forming the sides of the river valleys. The upper surface of this mass of moving ground water is indicated by the level of the water in superficial wells. This surface is not necessarily horizontal. It is in constant motion, travelling towards the outflow, and the rate of movement is governed by the porosity of the soil, slope, nature of outlet, etc. An intimate knowledge of the entire history of a water will often be necessary to an intelligent comprehension of certain analytical data.

CHAPTER II

THE PHYSICAL EXAMINATION OF WATER

THE physical examination comprises a determination of the turbidity, colour, odour, and taste.

Turbidity.—Pure waters are free from visible particles in suspension: the slightest degree of opacity should render a water suspicious. On the other hand, the most transparent and brilliant waters may contain the most pronounced pollution. Turbidity may be produced by access of the contents of cesspools, drains, manure heaps, and surface refuse of all types, especially after rains, when it forms often the worst kind of pollution. It may be produced by particles of clay, iron, chalk, etc., when a chemical and microscopical examination may be necessary to disclose the nature of the matter in suspension. Waters containing iron very often deepen in opacity during the first day or two after collection, owing to the formation of persalts of that metal, which are highly insoluble. Such opacity immediately disappears on the addition of a small quantity of dilute HCl.

Estimation of Turbidity.—Place the Winchester on a white porcelain tile in a good north light, and examine it carefully with the naked eye. Much information regarding opacity, sediment, etc., may thus be gained by a practised eye.

The sample may be described as brilliant (aeration good), clear, slightly turbid or opalescent, turbid, markedly turbid. To estimate the quantity of matter in suspension, filter 100 c.c. through a hard filter and evaporate in a platinum dish to dryness. The difference between the weight of the residue dried at 100° C. and that of 100 c.c. of the unfiltered sample similarly treated will represent the desired result. Or, where a centrifugal machine is available, by means of small tubes the sediment may be read off quantitatively on a gradu-

ated scale. The amount of light permitted to pass through a column of opalescent water mounted in a glass cylinder can be matched by the illumination of a polarized light ray passing through a second similar glass cylinder containing no water; the degree of rotation of the Nicol of the eyepiece expresses the degree of turbidity.

Colour.—Uncontaminated rain water presents a pale blue tint in the 'two-foot' tube. Yellow tints point to organic matter, brownish-red suggest a peaty origin, and reddish-yellow indicate iron. Any appreciable shade of yellow or brown will excite suspicion, and lead to a careful search for the cause. Colour tables have been formulated for the use of water analysts, but do not seriously assist a trained eye.

Clean thoroughly and fill the 'two-foot' tube; place it on the tile; look down through the column, noting the tint of colour, which may range from a pale sky-blue to a yellow or brown.

As to colour, for all ordinary purposes the naked-eye inspection is sufficient, but if for any reason great accuracy is required a tintometer may be used. Two hollow glass wedges containing respectively dilute solutions of CuSO_4 , and a mixture of ferric and cobalt chlorides slightly acidified, are made to slide over each other in front of an empty tube, so that any desired combination of blue and brown tints can be obtained. Alongside is placed a similar tube filled with the sample, and the wedges are arranged so that on looking down upon a white surface the colours exactly match. The prisms are graduated in millimetres, and the results are expressed in terms of millimetres of blue and brown.

Water may be variously coloured by algæ and other vegetable organisms. *Crenothrix polyspora* (rich in iron) colours it red or reddish-brown, and decomposing accumulations of the dead organism may produce serious nuisance. Green and blue algæ produce their respective tints, and peat, according to its concentration, all shades of brown.

Odour.—Drinking water should be free from all odour. Dissolved gases may be liberated by slightly warming the water, say to a temperature of 37°C . In the case of peaty waters it has been found at times that even after the most careful filtration a slight odour still attaches to the water. For many reasons peaty waters

do not furnish good supplies, and where other sources are available should be passed over.

River waters usually have a faint smell, due to a variety of causes, most often, perhaps, to vegetable organisms, some of which—*e.g.*, the well-known sewage fungus—are associated with the production of H_2S .

The dead and decomposing remains of plants and animals furnish a variety of odours, not only in river waters, but in cisterns, reservoirs, and mains. Of late years attention has been called to distinct species of lowly vegetable forms which produce disagreeable odours in water.

It is customary to obtain the sample of water for physical examination from the vessel containing that for the chemical examination, and something may now be said respecting the mode of collecting such samples. The so-called 'Winchester' quart bottle has long been used for this purpose, and when made of colourless glass answers the purpose admirably. The bottle should be thoroughly cleansed by rinsing with dilute HCl , and afterwards removing the last trace of acid with distilled water. Where it has to be sent by rail, etc., it is packed in a tightly-fitting wicker case or wooden box fitted with padlock. Before filling, the bottle should be rinsed with a portion of the sample. A little air space should be left under the stopper to avoid cracking of the neck through rise of temperature. Procedure in collection will vary according to the object of the examination. If it is desired to ascertain whether, for example, lead is dissolved by a water in the house-pipes, it will be necessary to collect the first runnings from the taps in the morning. When a bacteriological examination is required, a special method must be pursued, which will be described later. Where the sample is to be taken from a river, cistern, etc., the bottle, prepared as above, is usually immersed some little distance below the surface, where the stopper is removed and the bottle filled. A small portion is poured out in order to procure the air space mentioned, and the stopper inserted. Various forms of apparatus have been devised for collecting samples under different conditions, but the circumstances will in all cases suggest the mode of procedure, if it be kept in mind that a fair sample of the water as it is usually found is the object desired.

A correct record of the sampling process, etc., should be made on the spot, and attached as a label—

1. Date, time, and place of taking sample.
2. Depth below surface, state of water-level—high, low, or average.
3. Particulars of rainfall and of geological strata of district.
4. Depth of water-level below ground-level.
5. Description of surroundings, possible sources of pollution, such as sewers, cesspits, cemeteries, etc.

In many cases it is well for the analyst to supply his own collecting-bottle, with instructions for taking the sample and filling up the label. The sender should be made to understand that the specimen must be of the same nature exactly as that actually consumed, and that it is desired to ascertain the maximum degree of pollution that may at any time obtain. All such particulars, as also the results of the analysis, should be transcribed into a book and preserved for future reference.

Estimation of Odour.—Place 250 c.c. in a stoppered flask, and heat to 37° C. in an air-bath. Remove the stopper and smell. It is generally sufficient to shake the sample well in the cold, rapidly remove the stopper, and smell. The variety of odours is infinite. Many odours are produced by organisms, either as products of their life-history or of their death and putrefaction. *Beggiatoa*, *Chara*, and certain species of *Crenothrix* produce an offensive odour of H_2S . It is believed that *Beggiatoa* during its life-cycle reduces sulphates, and produces under favourable circumstances large quantities of H_2S . *Crenothrix*, moreover, often produces abundance of colour, varying from brown to red. *Tabellaria*, *Meridion*, and certain diatoms, as also the protozoon *Cryptomonas*, furnish a distinctly aromatic odour. A fishy odour is produced by *Volvox* and the protozoa *Glenodinium*, *Bursaria*, and *Uroglena*. A grassy odour accompanies *Rivularia*, *Anabæna*, and *Cælosphærium*.

Taste.—Pure rain water well aerated has a fairly distinctive taste, more easily appreciated than described. So also have peaty waters, sea water, and chalybeate waters. The taste of a particular sample may be, however, everything to be desired, whilst the water is the foulest of the foul. Taste, however, is of little service to the analyst, and not always to be recommended. Iron is about the

only ingredient that can in this way be detected in very small quantities, being recognisable to the amount of 0·5 part per 100,000.

Potable waters have been classified as—

Wholesome.—(1) Spring water; (2) deep-well water; (3) upland surface water.

Suspicious.—(4) Stored rain water; (5) surface water from cultivated land.

Dangerous.—(6) River water polluted with sewage; (7) shallow-well water.

CHAPTER III

THE CHEMICAL EXAMINATION OF WATER

It will be well for the student from the first to fit up his own apparatus, and make his own standard solutions. He must learn to use properly the chemical balance, and a special demonstration is devoted to the mechanism, methods of adjusting and using this all-important instrument. Before commencing to weigh, he should see that the balance is accurately levelled, and that the index moves without effort over the whole field of the graduated scale, and comes to rest at zero. All weights, basins, etc., should be transferred to and from the scale-pans only when these are supported. It is customary to use three rows of weights, grammes (brass), decigrammes and centigrammes (platinum), and milligrammes (platinum). A rider of platinum applied to the beam also reads milligrammes. The right-hand pan should be used only for weights, and these should be placed methodically in three rows in front of the operator. By this means the total reading is most easily obtained and checked.

Immediately on finishing a weighing all weights should be transferred to the box, with the forceps used for the purpose, and the box and balance carefully closed.

The standard solutions in use in water analysis are of two types:

1. Normal, decinormal, centinormal, etc.
2. Standards of such strength that a litre contains the equivalent of a gramme, or submultiple of a gramme, of the substance to be estimated.

A standard solution is said to be normal when one litre contains the equivalent weight in grammes of an element, acid, alkali, or salt.

The molecular weight of HCl is 36.35; therefore 36.35 grammes HCl per litre = normal HCl , written N.HCl .

In like manner $\text{N.NaOH} = 40$ grammes per litre.

Since the term 'equivalent' signifies the weight in grammes of the substance under consideration, which is chemically equivalent to 1 gramme of H, normal $\text{H}_2\text{SO}_4 = \frac{98}{2} = 49$ grammes per litre.

A decinormal solution ($\frac{\text{N}}{10}$) is one-tenth the strength of a normal—thus $\frac{\text{N}}{10} \text{NaOH} = 4$ grammes per litre—and a seminormal ($\frac{\text{N}}{2}$) and centinormal ($\frac{\text{N}}{100}$) are respectively one-half and one-hundredth the strength of the normal—viz., 20 grammes and 0.4 gramme per litre respectively.

In tribasic acids one-third of the molecular weight in grammes per litre constitutes a normal solution, and so on for acids of higher basicity.

The terms 'normal,' 'decinormal,' etc., are used sometimes with a different meaning. Permanganate of potassium, as we shall see presently, in acid solution is reduced by many substances, according to the equation $\text{K}_2\text{Mn}_2\text{O}_8 = \text{K}_2\text{O} + 2\text{MnO} + \text{O}_5$, in which 2 gramme molecules of KMnO_4 correspond to 5 gramme molecules of oxygen or to 10 gramme molecules of hydrogen. Accordingly, in order to put permanganate of potassium on a hydrogen basis, a normal solution is made to contain $\frac{316.3}{10} \left(\frac{2\text{KMnO}_4}{10} \right) = 31.63$ grammes per litre. In the same way $\text{K}_2\text{Cr}_2\text{O}_7$, which in acid solution parts with O_3 , requires for a normal solution $\frac{294.5}{6} \left(\frac{\text{K}_2\text{Cr}_2\text{O}_7}{6} \right) = 49.1$ grammes per litre.

The second type of standard solution used is constructed so that a minimum amount of calculation suffices in estimating results. Since it is customary to represent the various items of the analysis as parts by weight per 100,000 of the water, and since 1 c.c. of water weighs 1 gramme (1,000 milligrammes), 100 c.c. of water will weigh 100,000 milligrammes.

It is therefore convenient, when possible, to work on 100 c.c. of the water sample throughout the various estimations, and to use a standard solution that will give readings directly in the above terms.

Suppose we wish to estimate the quantity of Cl in a water, we use a solution of AgNO_3 of such strength that 1 c.c. is equivalent

to 1 milligramme Cl. To make this solution we refer to the molecular weight of AgNO_3 , and the atomic weight of Cl. $\text{AgNO}_3 + \text{NaCl} = \text{AgCl} + \text{NaNO}_3$.

170 grammes AgNO_3 precipitate 35.35 grammes Cl.

\therefore dividing by 35.35, we find that 4.8 grammes AgNO_3 precipitate 1 gramme Cl.

If, then, we dissolve 4.8 grammes AgNO_3 in 1 litre of water we obtain a solution 1 c.c. of which precipitates 1 milligramme of Cl, and working with 100 c.c. of water, the number of c.c. of the silver nitrate solution used indicates the number of milligrammes of Cl in 100,000 milligrammes of the water, which is parts per 100,000.

Again, in estimating NH_3 a standard solution of NH_4Cl is prepared and used in the same way.

One molecule of NH_4Cl contains 1 molecule of NH_3 .

| | | | |
|---------------|---|--------------------|----------|
| 53.35 grammes | „ | contain 17 grammes | „ |
| and 3.14 | „ | „ | 1 gramme |

Therefore a litre containing 3.14 grammes NH_4Cl will contain 1 gramme NH_3 , and consequently 1 c.c. contains 1 milligramme. It is found convenient to dilute this 100 times, so that 1 c.c. = 0.01 milligramme NH_3 .

Standard solutions should be stored in bottles in such manner that both internal and external evaporation are impossible. In the first case, where the bottle is not quite full, pure water will evaporate and condense on the upper portions of the vessel; in the second, evaporation will take place into the atmosphere. The loss of water will naturally depend on the substance dissolved, the temperature, the age of the solution, and the frequency with which it is used. A rough estimate may often be made of the probable amount of change in strength by noting the date of preparation, which should always be found on the label. Some standards undergo chemical change by the action of light, and should therefore be kept in the dark.

In reading a burette, arrange it so that the lower convex line of the meniscus is in the same horizontal plane with the eye; the

division of the scale cut by the lowest point of this convex line is the reading.

In measuring small quantities of liquids much time may be saved by using a few plain 10 c.c. pipettes graduated to tenths of a c.c., and for quantities under a c.c. a 1 c.c. pipette graduated to hundredths. These can be easily and rapidly cleaned, and as easily and rapidly manipulated, and may often take the place of burettes. In weighing platinum and porcelain basins, crucibles, etc., it is very necessary to see that they are quite dry. To insure this, especially after heating, they should be placed for ten minutes in a desiccator immediately before going to the balance. It is also necessary to be certain that all such vessels are thoroughly clean. Accurate notes of all operations, measurements, weights, etc., should be made in the bench notebook, and considered as much a part of the work as the operations themselves. Without this notebook it is impossible to get on with analytical chemistry. Where possible it is well to write down the chemical equations representing decompositions. When in doubt in this matter refer to a work on chemistry. All colour matches are best made in glass cylinders standing on a white ground, as the operator faces a north light.

The Reaction of Water.—This is an important item, and should form the first step in the routine chemical examination. In addition to the use of red and blue litmus-papers, it is often well to use a more delicate indicator, such as phenolphthalein, and to estimate the amount of acidity (when acid) in 100 c.c. by titrating with $\frac{N}{10}$ NaOH, or of alkalinity (when alkaline) with $\frac{N}{10}$ H_2SO_4 . An acid water dissolves lead, iron, and zinc; it also fixes ammonia, and so prevents its being distilled off. Some hold that neutral waters and those possessing very slight temporary hardness are capable of dissolving lead. It should be remembered, however, that sodium carbonate when present prevents this action. Houston has correlated the acidity and plumbo-solvency of a large number of moorland waters.

He causes the sample to percolate upwards through a column of specially prepared lead shot at a uniform rate. He then collects successive 50 c.c.'s, and estimates the amount of lead in each.

The following figures are taken from a report to the L.G.B.:

| ACIDITY. | | PLUMBO-SOLVENCY. | |
|----------------|--|-----------------------------|-------------------------------|
| Number of c.c. | | Mgms. of Pb in | |
| 10 | $\text{N Na}_2\text{CO}_3$ required to neutralize 100 c.c. of the Water. | 100 c.c. of the Water after | Filtration through Lead Shot. |
| | 0.2 | | 0.28 |
| | 0.3 | | 0.25 |
| | 0.4 | | 0.4 |
| | 0.5 | | 0.66 |
| | 0.6 | | 0.92 |
| | 0.8 | | 1.55 |
| | 0.95 | | 2.66 |
| | 1.5 | | 2.8 |
| | 1.7 | | 5.6 |
| | 2.2 | | 8.6 |

Some waters not acid, and failing to dissolve lead, exert an 'erosive' action, forming an insoluble film of oxyhydrate upon the lead, which after a time may become detached, and produce a degree of opacity.

Chlorides in Water.—Free Cl rarely occurs in water-supplies. Certain manufacturing effluents may on occasion contain small quantities of free Cl, but the quantity is so small and the occurrence so rare that this form of Cl may be practically ignored. The great bulk of Cl in drinking water is found as NaCl. All soils and sub-soils contain this salt in large amounts. The water-bearing strata are rich in chlorides, especially NaCl, and consequently rain water (which itself may contain as much as 0.5 part per 100,000 NaCl), as it percolates from the surface to the impermeable stratum on which it rests, dissolves these in considerable quantities. CaCl_2 and MgCl_2 are found in certain strata—chalk and limestone—in much smaller quantities, but MgCl_2 abounds in sea water, and in large quantity is distinctive of it. Wells, reservoirs, etc., to which sea water can obtain access will yield waters rich in MgCl_2 . Sources of water subject to much evaporation, especially if situated near the sea, exhibit large quantities of chlorides. The total Cl in sea water approaches 2,000 parts per 100,000, and if this figure be kept in memory it will explain the large estimations often found some considerable distance from the littoral. During the passage of water through the soil, subsoil, and strata, Cl is not likely to be diminished as are the organic matter and bacteria.

When we have accounted for all the Cl contributed by rain water,

sea water, soil, subsoil, and strata, and trade effluents from chemical works, paper factories, etc., there may remain a surplus furnished by organic pollution of animal origin. This surplus is of some import to the analyst, as indicating sewage; but before it is returned as such all the possible sources of origin just mentioned must be rigidly excluded. Vegetable organic matter does not yield this surplus Cl. Attempts have been made in U.S.A. to estimate and permanently record the Cl due to the natural causes named, so that sewage pollution may be readily detected. Maps have been constructed and points furnishing equal quantities of Cl joined by lines named 'isochlors.' In districts remote from the sea, and centres of population and land cultivation, such maps may be more or less reliable, but in this country they would be useless. Whilst it is true that animal pollution contains much Cl (urine about 1 per cent. chlorides), and that soils, strata, etc., in certain districts yield fairly constant quantities, still there are variations in many localities in these natural sources, and it is only where large quantities of sewage have gained access to waters that we can rely on the surplus Cl as evidence of this accession. In the case of small amounts of sewage this surplus Cl figure is of little if any value. But in a water analysis the most important information lies very often not so much in the exact amount of a particular constituent as in the fact that its presence points to past pollution, and consequently to the possibility and even probability of a recurrence of such pollution. In this light Cl and nitrates play an important rôle. These afford unmistakable evidence of previous contamination; they are the distinct and unchangeable indications of previous pollution, but as to whether recent or remote they indicate nothing. Hence the necessity for further and different forms of examination. As to the amounts of chlorides that should condemn waters, it is difficult to speak, since there is such infinite variety in the quantities contained in different soils and strata. $MgCl_2$ and $CaCl_2$ render waters hard, so that more than 4 or 5 parts of either or both of these per 100,000 will cause a large destruction of soap, and these figures will in most cases form the limit for domestic waters. NaCl may go up to perhaps 50 parts per 100,000; above this it imparts a taste, and the water consequently will not be fit for drinking.

Estimation of Cl.

APPARATUS AND REAGENTS REQUIRED.

A white porcelain basin capable of holding 250 c.c.

A glass stirring-rod.

A burette charged with standard solution of AgNO_3 , of which 1 c.c. is equivalent to 1 milligramme Cl (4.8 grammes AgNO_3 to a litre of water).

A 5 per cent. solution of K_2CrO_4 .

Place 100 c.c. of the water in the porcelain dish.

Add 1 c.c. of the K_2CrO_4 solution, and stir.

Run in from the burette drop by drop the silver nitrate solution until the pale yellow colour remains permanently orange.

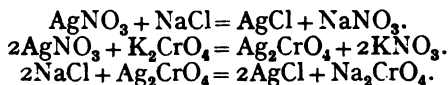
Take the reading.

The rationale of the process is as follows:

AgNO_3 , when added to a solution of chlorides, forms AgCl , a white curdy precipitate insoluble in HNO_3 , soluble in NH_4HO . Without a special indicator it would be impossible to determine when the whole of this white precipitate had been formed—when the whole of the Cl had been deposited.

K_2CrO_4 is also acted on by AgNO_3 , and Ag_2CrO_4 formed, which is red. But so long as any chloride remains unacted with Ag, the silver chromate is decomposed and AgCl formed; hence the disappearance of the red colour on stirring. Immediately the whole of the Cl is precipitated as AgCl the red silver chromate remains.

The reactions are represented by the equations—



It is obvious that the K_2CrO_4 should be free from Cl. Acidity in the water will dissolve Ag_2CrO_4 ; hence if a water is even slightly acid it must be neutralized. Freshly precipitated CaCO_3 is the best alkali to use, and it should be used only to the point of neutralization. If too little K_2CrO_4 is used the Cl reading will be too high, and if too much be used it is difficult to determine the end; 1 c.c., accordingly, is found a suitable quantity when the solution is of the above

strength. It will be noticed that as the titration proceeds the red Ag_2CrO_4 disappears more slowly on stirring, until finally it ceases to disappear. This is explained by the continuous decrease in the original chloride. Whilst abundance of this undecomposed chloride remains in solution, the Ag_2CrO_4 is rapidly robbed of its Ag and the red colour discharged; but as the chloride diminishes and the end approaches, the decomposition of the Ag_2CrO_4 becomes slower and slower, until at the end of the reaction it ceases, and the red Ag_2CrO_4 permanently remains.

Since the colour-change from pale yellow to red is somewhat difficult to detect in daylight (it is more easily perceived by gas-light), a flat glass cell whose plates are $\frac{3}{8}$ inch apart should be filled with chromate solution of the same tint as that of the contents of the basin, and interposed between the eye and the basin during titration, when the appearance of the red silver chromate becomes strikingly manifest. The effect is to neutralize the yellow and to cause the appearance of the basin to be the same as if it were filled with pure water. In working with turmeric, cochineal, etc., cells should be used filled with corresponding solutions of turmeric, cochineal, etc.

The number of c.c. of silver nitrate run in represents the number of parts of Cl per 100,000.

$$\begin{aligned} 100 \text{ c.c. water} &= 100,000 \text{ milligrammes,} \\ 1 \text{ c.c. AgNO}_3 &= 1 \text{ milligramme Cl;} \\ \therefore \text{ the number of c.c. AgNO}_3 \text{ used} &= \text{number of parts Cl} \\ &\text{per 100,000 water.} \end{aligned}$$

Some operators subtract .1 c.c. from the AgNO_3 figure as the quantity required to form the slight permanent orange colour. Others add a small measured quantity of the water sample from a burette until the permanent orange tint departs, and reckon half of this with the AgNO_3 reading.

It is well always to do two careful estimations, and take the mean. When once an idea of the quantity of Cl present is obtained, two careful estimations can be performed very rapidly. A control basin containing 100 c.c. of the same water and 1 c.c. of K_2CrO_4 may assist in determining the end reaction.

Where small quantities of Cl are to be estimated, 250 c.c. or 500 c.c.

of the water may be concentrated by evaporation to 100 c.c. Alkaline silicates, nitrates, and phosphates slightly affect the Cl estimation, but not to such a degree as to require correction.

Chlorine is sometimes returned in terms of sodium chloride. This figure is found by multiplying the Cl return by $\frac{58.35}{35.35}$. Where CaCl_2 , or MgCl_2 , or both, enter into the problem, corrections have to be made in accordance with the respective molecular weights and the quantities of each present.

In chalk and red sandstone waters 3 parts of Cl per 100,000 may occasion no suspicions of sewage, and 4 or 5 parts may be passed, unless organic pollution is indicated by other items of the analysis. Pure surface waters seldom contain more than 1 part per 100,000, whilst deep greensand waters may give rise to 15 to 20 parts per 100,000, and still be absolutely pure.

The following are a few examples of the Cl figures for different waters:

| | | | | | Parts
per 100,000. |
|---|---|---|---|---|-----------------------|
| A well in St. Pancras | - | - | - | - | 4.5 |
| Lambeth water-supply | - | - | - | - | 1.9 |
| | - | - | - | - | 2.0 |
| Southwark "water-supply" | - | - | - | - | 1.85 |
| A well in Devonshire | - | - | - | - | 3.1 |
| Thames water at Waterloo Bridge | - | - | - | - | 103.2 |
| Deep well near Hindhead | - | - | - | - | 112.3 |
| Sample of rain water taken from rain gauge in Herts | - | - | - | - | 0.3 |

Hardness.

The hardness (soap-precipitating power) of a water exerts little influence on health, but from an economic point of view is of some importance.

A soap is a chemical salt formed by the union of an inorganic base with one or more fatty acids.

Sodium and potassium soaps are soluble in water, and when shaken with it form a dense froth or lather. Calcium and magnesium soaps are insoluble in water, and fail to form a lather. Hence, if a solution of a soluble soap be added to water containing calcium or magnesium salts, these last will be completely precipitated in the form of insoluble calcium or magnesium soaps before a lather is produced. Accordingly, by using a standard soap solution, an

approximate estimate of the quantity of such soap-precipitating bodies in a water can be made. The total quantity of such bodies, as measured by the standard soap solution, constitutes the total hardness. Other bodies than calcium and magnesium salts are occasionally present in water, which act in a similar manner on soap. If much sodium chloride be present, it will precipitate soap from its solution in an unaltered state.ⁱ

CaCO_3 and MgCO_3 , especially the first, have by far the greatest share in rendering waters hard. These salts are formed in solution in the soil as bicarbonates [$\text{Ca}(\text{HCO}_3)_2$ and $\text{Mg}(\text{HCO}_3)_2$] by CO_2 dissolved in rain water. On boiling such waters, CO_2 escapes, and insoluble carbonates separate out as a precipitate—



The addition of slaked lime to water containing the bicarbonates of the alkaline earths results in the precipitation of the lime added and the bicarbonates thus:



If now the boiled water be filtered, made up to its original volume with distilled water, and again titrated with standard soap solution, the permanent hardness is obtained.

The difference between the total and the permanent hardness is the temporary hardness.

The soap test has been made to measure the quantity of CaCO_3 and other salts which produce hardness, but this is not accurate quantitative analysis. It should be clearly understood that the chemical action is multiple and indefinite, and altogether different from that which usually takes place, when in quantitative analysis we titrate one definite compound against another. All that can be claimed for the soap process is that it indicates the amount of soap-destroying bodies present in a given water, but fails to form a measure for any in particular.

The following compounds produce hardness:

CaCO_3 , MgCO_3 , CO_2 in solution, CaSO_4 , MgSO_4 , Fe_2O_3 , and other Ferroxides, zinc salts, SiO_2 , $\text{Al}_2(\text{OH})_6$, chlorides, nitrates, phosphates, and free mineral and organic acids.

The temporary hardness, which is got rid of by boiling, is for

the most part produced by CaCO_3 and MgCO_3 , held in solution by CO_2 . After these come small quantities of CaSO_4 and MgSO_4 , which are also thrown out immediately CO_2 is driven off, but the great bulk of these sulphates remains in solution. Lastly, in a few cases minute quantities of oxides of Fe, silica, and alumina are deposited. Phosphate of Ca, if present in appreciable quantity, may, under certain conditions, be deposited in very small amounts. On cooling some of the precipitated MgCO_3 , and to a less degree CaCO_3 , CaSO_4 , and $\text{Ca}_3(\text{PO}_4)_2$ will redissolve and go to form permanent hardness.

MgCO_3 destroys nearly 50 per cent. more soap than CaCO_3 , but is found in potable waters in very much less quantity.

Estimation of Hardness.—Prepare a standard solution of calcium chloride in the following manner: Weigh accurately 0.2 gramme pure calcite (CaCO_3), and dissolve it in dilute HCl , taking care to keep the vessel covered so as to avoid loss by spiriting. Evaporate this solution to dryness on the water-bath. Add water, and again evaporate to dryness, and repeat these processes in order to remove all free hydrochloric acid. Now dissolve the residue of neutral CaCl_2 in water and make up to a litre. One c.c. = the equivalent of 0.2 milligramme CaCO_3 . In other words, this solution possesses hardness = 20 parts per 100,000.

Prepare a standard soap solution by dissolving about 13 grammes of Castile soap in a litre of equal parts methylated spirit and water. Stand in a cool place for some hours, and filter.

The titration and dilution of this soap solution is carried out as follows:

Make up 50 c.c. of the calcium chloride solution to 100 c.c. with distilled water (10 parts hardness per 100,000), and place in a stoppered bottle of 250 c.c. capacity. Run in from a burette, 1 c.c. at a time, the soap solution. Close the bottle, and shake vigorously for a short period until a lather remains on the surface as an unbroken layer for five minutes. Towards the end of this operation the amount of soap solution added should be lessened, and finally should not exceed $\frac{1}{2}$ c.c. As the end is reached, the sound and shock produced by shaking becomes much more gentle.

The student should carefully prepare a number of similar lathers by shaking 100 c.c. distilled water in a similar bottle, and note exactly the amount of soap solution required. This quantity

will be found to be about 1 c.c. of the finished standard soap solution.

In the present case the quantity of soap solution used should be 11 c.c. (10 c.c. to precipitate the equivalent of 10 milligrammes of CaCO_3 , and 1 c.c. to produce the lather). Suppose, however, that 9 c.c. soap solution be found sufficient to produce the characteristic lather, it is evident that the solution must be diluted with aqueous spirit in the proportion of 9 to 11. Dilute, therefore, 900 c.c., or thereabouts, of the original litre to the volume $\frac{900 \times 11}{9}$ c.c., and keep the remainder for fortifying the standard, as in time it loses strength, especially when, on keeping, it becomes turbid. Label the solution Standard Soap 1 c.c. = 1 milligramme CaCO_3 .

Should the soap solution prove too weak, it must have additional soap added and be put through the same process of standardization until found correct.

A standard soap solution may be prepared in another way: Dissolve 80 grammes chemically pure oleic acid in alcohol, add a few drops phenolphthalein and a strong solution of KOH in alcohol, until the oleic acid is neutralized and saponification therefore complete (the liquid retains the faintest purple colour); then titrate with the calcium chloride solution, and dilute to standard strength.

The following is an example of the determination of the hardness of a sample of a London (New River) water:

Take 100 c.c. of the water in a 200-c.c. stoppered bottle. Fill a 50-c.c. burette mounted on a stand with standard soap solution (1 c.c. = 1 milligramme CaCO_3). Run in the soap solution 1 c.c. at a time, shaking vigorously after each addition, until a permanent lather remains unbroken for five minutes when the bottle is laid on its side. As the end of the reaction approaches, the hard metallic sound at first heard on shaking gives place to a dull thud, the froth which previously disappeared almost instantaneously remains, and adheres in specks to the sides of the bottle.

Twenty-one c.c. of standard soap solution were required in this case to complete the titration. Subtracting 1 c.c. used in producing the lather, we find that 20 c.c. were precipitated by the 100 c.c. of water.

But each c.c. = 1 milligramme CaCO_3 ;

\therefore 20 c.c. = 20 milligrammes CaCO_3 .

and 100 c.c. of this water contains 20 milligrammes of soap-precipitating substances, or a 'total' hardness equal to 20 parts per 100,000. In waters containing magnesium salts the lather is slowly produced, and of a dirty, granular appearance, very unlike the light frothy condition seen in hard waters destitute of Mg salts.

To obtain the 'permanent' hardness in the above example, place 100 c.c. in a small beaker on a porcelain ring over a Bunsen flame, and boil for fifteen minutes, or till one-third of the volume has evaporated. Filter into a clean 100-c.c. flask, and make up to the mark with distilled water. Transfer to the stoppered bottle and determine the hardness as above: this is 'permanent hardness.'

13.5 c.c. of the soap solution were required to lather the 100 c.c. of water prepared as described.

$$13.5 \text{ c.c.} - 1 \text{ c.c.} = 12.5 \text{ c.c.},$$

or 12.5 parts permanent hardness per 100,000.

The 'temporary hardness' = difference between 'total' and 'permanent' hardness.

$$20 - 12.5 = 7.5 \text{ parts temporary hardness per 100,000.}$$

Hard waters, whilst palatable, cause waste of soaps, and fail somewhat in cooking vegetables, meats, etc., and in making infusions of tea and coffee. They are unsuitable for boilers, in that a deposit forms on the interiors which by reason of its low conductivity of heat wastes fuel, and from its divergent coefficient of expansion may lead to explosions.

This deposit or crust will consist of bodies representing both temporary and permanent hardness. Carbonates of Ca and Mg will fall out first, and be followed by their sulphates, together with salts of iron, silica, and alumina.

In this country the hardest waters arise from the chalk, dolomite, and new red sandstone strata, carbonates of Ca and Mg forming by far the largest proportions of soap-destroying compounds.

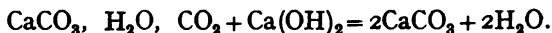
Where the hardness exceeds 20 parts per 100,000, it is well in performing the estimation to dilute the sample with an equal bulk of distilled water. The total hardness of a potable water should not exceed 25 to 30 parts per 100,000. Waters whose hardness falls below 10 parts are considered soft, whilst those containing 20 to 30 parts are hard, and upwards of 30 parts very hard.

Clark's scale of degrees represents hardness as grains per gallon (parts per 70,000).

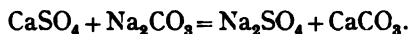
It is universally agreed that a good water should contain less than 10 parts per 100,000 of permanent hardness, and of this little should be due to magnesium salts.

Temporary hardness can be easily got rid of; not so permanent.

In Clark's process, as noted above, slaked lime is used for softening—in other words, for combining with the CO_2 in solution, thereby causing insoluble carbonates to separate out which were previously held in solution by the CO_2 . Care should be taken that no excess of lime is added.



Softening of permanent hardness may be effected by the use of Na_2CO_3 :



Clark's method does not yield accurate results if a large quantity of Mg salts is present. These salts do not materially affect the process now to be described.

Estimation of Hardness by Standard Acid.—Determine first the temporary hardness by titrating the calcium and magnesium salts which form it with $\frac{N}{10} \text{H}_2\text{SO}_4$, using methyl orange (the sodium salt of a colour acid which is not interfered with by CO_2) as indicator.

Add to $\frac{1}{2}$ litre of the water, or less if it be very hard, 4 or 5 drops of methyl orange solution, and run in $\frac{N}{10} \text{H}_2\text{SO}_4$ from a burette until the colour changes pink. Calculate the weight of CaCO_3 from the number of c.c. of acid used and convert this into parts per 100,000.

Example.—500 c.c. water required 9 c.c. decinormal sulphuric acid.

$$\begin{aligned} 1 \text{ c.c. } \frac{N}{10} \text{H}_2\text{SO}_4 &= 1 \text{ c.c. } \frac{N}{10} \text{CaCO}_3 = 0.005 \text{ gramme CaCO}_3; \\ \therefore 500 \text{ c.c. water} &= 0.005 \times 9 \text{ grammes CaCO}_3; \\ \therefore 100 \text{ c.c. water} &= 0.001 \times 9 \quad \quad \quad \text{,,} \quad \text{,,} \\ &= 0.009 \quad \quad \quad \text{,,} \quad \text{,,} \\ &= 9 \text{ milligrammes CaCO}_3. \end{aligned}$$

Hence the temporary hardness in terms of $\text{CaCO}_3 = 9$ parts per 100,000.

Permanent Hardness.—To 250 c.c. water add excess $\frac{N}{10} \text{Na}_2\text{CO}_3$, say, 50 c.c., and boil for half an hour. Should Mg salts be present,

Solid Residue.

The total solids in waters vary greatly in extent, ranging from 2 to 3 parts in rain water to over 3,000 parts in sea water per 100,000. From the purely health point of view, perhaps little information will be derived from an estimation of the total solids. Occasions, however, arise in which it may be desirable to estimate the total solids, and also the quantities of certain constituents, such as Ca and Mg salts. Where these latter exist in large quantity in the form of sulphates, it is found that the waters are unfit for drinking, from their action on the alimentary tract. The incineration of the dry residue affords a check on some of the other portions of the analysis dealing with organic matter. On the whole, any useful information that can be obtained will, for the most part, centre round the quantities of Ca and Mg salts present, especially the sulphates.

Chalk waters contain little sulphates; limestone waters hold chiefly CaSO_4 , at times to the extent of 15 to 20 parts per 100,000; magnesium limestone (dolomite) generally contains much less CaSO_4 and considerable MgSO_4 . Sulphates in water are chiefly derived from strata; a very small amount is due to the oxidation of S in organic matter; a small quantity in the rain water of large towns has its origin in the solution of the oxides of S found in the atmosphere; whilst in exceptional cases an appreciable portion is due to the oxidation of metallic sulphides.

Phosphates in marked quantities indicate organic pollution, especially urine. The phosphates of the alkalies are those chiefly found in water. But, in that certain geological beds and organic matter of purely vegetable origin contain phosphates, no very direct information is obtained from their estimation, and it is usually unnecessary to go beyond a qualitative examination.

In a few instances silica may require to be estimated: this compound lessens the plumbo-solvency of water.

In clear waters the solids are all in solution; in turbid waters they are partly in solution and partly in suspension. It is customary to estimate the solids in solution, but as this requires complete sedimentation it may be advisable in cases where time is limited to perform the estimation on the sample after thoroughly

shaking. The method adopted, however, should be stated on the report.

Measure out 100 c.c. of the water, and place in a clean platinum basin on a water-bath, 25 c.c. at a time, as evaporation proceeds. When dry, transfer the basin, after carefully wiping the outside, to an air-bath at 37° C. for half an hour. Remove to a desiccator for ten minutes, and weigh. By drying at this low temperature no water of crystallization is lost, and no decomposition takes place. Further drying should be effected, if necessary, until a constant weight is obtained. This weight, less that of the dish, represents the total solids.

With platinum-tipped tongs hold the dish over a Bunsen flame until thorough incineration is effected. After cooling in the desiccator, weigh again to obtain the non-volatile solids. The difference between this last and the previous weight represents the volatile solids. The degree of charring (organic matter) which occurs during incineration should be noted; also the smell—odour of burnt sugar indicates vegetable matter, burnt horn animal substance.

Ca.—Where it is deemed necessary to estimate the quantity of Ca salts, Mg salts, or both, 500 c.c. of the sample should be evaporated down to 200 c.c., and the Ca removed by precipitation with $(\text{NH}_4)\text{HO}$, NH_4Cl , and $(\text{NH}_4)_2\text{C}_2\text{O}_4$. The precipitate of CaC_2O_4 , when thoroughly washed, dried, ignited, and weighed, represents the Ca as CaCO_3 , 56 per cent. of which is Ca. The weight of the crucible and ash of filter-paper must be accurately known and accounted for. It is well to let the beaker or other vessel containing the mixture of precipitate and fluid stand for some hours in a warm place, by which filtration is rendered much more easy and thorough. The student may be reminded that the addition of NH_4Cl holds Mg salts in solution.

Mg.—Concentrate the filtrate down to one-fifth its bulk or less. Add slight excess of sodium phosphate, and stand aside in a warm place for some hours. Filter, wash the precipitate well with dilute $(\text{NH}_4)\text{HO}$, dry, ignite, and weigh as $\text{Mg}_2\text{P}_2\text{O}_7$ (magnesium pyrophosphate). The Mg forms $\frac{8}{37}$ of this weight.

Phosphates.—It is rarely necessary to estimate phosphates. Where, however, required, proceed as follows:

Evaporate 200 c.c. of the water to dryness. Moisten the residue

with a few drops of pure HNO_3 and evaporate again to dryness, in order to render insoluble any silica that may be present. Dissolve in dilute HNO_3 and filter. Add ammonium molybdate in slight excess; keep, if possible, in a warm place over night, and filter. Wash the precipitate well with hot water, and dissolve in ammonia. Add a few drops NH_4Cl and slight excess of MgCl_2 , and filter. Wash the precipitate thoroughly with dilute ammonia. Dry, ignite, and weigh the $\text{Mg}_2\text{P}_2\text{O}_7$. The phosphates returned in the form of P_2O_5 will be represented by $\frac{71}{111}$ of this weight.

It is hardly necessary to say that a qualitative test for phosphates should be carefully performed before entering on the more lengthy quantitative estimation. For this test concentrate by evaporation a quantity of the water—say 200 c.c.—to one-tenth its bulk. To 10 c.c. in a test-tube add a drop or two of HNO_3 , 1 or 2 c.c. solution of ammonium molybdate, and heat to a temperature somewhat below boiling, for several minutes if necessary. A greenish-yellow coloration indicates traces of phosphates, a canary-yellow colour an appreciable amount, and a yellow precipitate larger quantities.

Silica.—This compound generally exists in water, either as soluble silicates of the alkalis, or as insoluble silicate of alumina. Evaporate 300 c.c. of the water to dryness after acidulating with HCl . Treat the residue with strong HCl , and transfer by washing to a filter with boiling water. Dry, ignite, and repeat the foregoing treatment with acid and boiling water three or four times. Finally dry, ignite, and weigh as SiO_2 .

Sulphates are readily detected by concentrating to about one-tenth, and adding to the warmed sample a drop of HCl and a few drops of BaCl_2 in solution, when a white insoluble precipitate of BaSO_4 is formed and rapidly sinks to the bottom of the test-tube. The insolubility of this precipitate should always be tested with sufficient strong nitric acid.

Estimation of Sulphates.—A measured quantity of the water is heated to boiling in a beaker; a few drops of HCl are added, and sufficient hot solution of BaCl_2 to precipitate the whole of the sulphates run in. Time is given to the precipitate to settle, and a little more of the BaCl_2 allowed to fall into the supernatant clear solution. If no turbidity is produced the reaction is complete;

but if even the slightest turbidity occur more BaCl_2 must be added, and the mixture again allowed to settle, until the addition of a drop of BaCl_2 produces no turbidity. The white precipitate is collected on a filter-paper, the weight of whose ash is known, well dried, ignited in a crucible, and weighed as BaSO_4 .

The SO_4 is returned as $\frac{96}{233}$ of this weight.

Alkaline phosphates, sulphates, and chlorides may indicate animal organic matter, especially urine, but it is often difficult to attribute to these salts a source in recent pollution, as all are found in strata free from organic matter. Where marked excess is found, the composition of the geological strata accurately known, and where frequent analysis of pure waters from the same strata are made, an increase of any or all may be attributed to organic pollution. But it should be remembered that slight variation in amount of these salts is met with from time to time in waters arising in certain strata, where contamination is out of the question.

Nitrites, nitrates, and poisonous metals, when present, will be found in the dry residue forming the total solids. The metals are most easily detected in this residue.

Poisonous Metals.

There are only a few metals whose compounds are found in water-supplies. Lead and copper are the chief; occasionally iron and zinc occur; and very rarely chromium and tin.

A qualitative examination should be performed in all cases for each of these metals; and where a possibility of other metallic compounds derived from mines, industrial wastes, etc., exists, a further careful investigation is necessary.

Lead.—Waters possessing an acid reaction, such as those derived from peaty moorlands, in which organic acids (ulmic, geic, etc.) are formed by certain micro-organisms, dissolve lead. The primary action of water on lead is an oxidation. In alkaline and strictly neutral waters the coating of oxide remains intact, but in acid waters it dissolves. Hard waters containing abundant carbonates form an insoluble oxycarbonate; hence hard waters lack the property of dissolving lead. Houston distinguishes between the solvent action of acid waters, and the 'erosive' action of neutral waters containing dissolved oxygen. Acid waters should be cut

out of public supplies, if they cannot be passed through chalk, limestone, etc., so as to be completely neutralized. Four parts of CaCO_3 or MgCO_3 per 100,000 are necessary to eliminate plumbo-solvency.

The effects of acid moorland waters on lead have been only too clearly seen in certain districts of Yorkshire and Lancashire, where the inhabitants have suffered from anæmia, constipation, colic, wrist-drop, depression, gout, renal disease, and other classical effects of lead-poisoning. The lead is dissolved out of materials of joints, block-tin pipes, house pipes, cisterns, etc.

Whilst carbonates and sulphates in water diminish plumbo-solvency, nitrates favour it, as lead nitrate is the most soluble salt of the metal. A rise in temperature up to 48° to 50°C. increases plumbo-solvency.

Compounds of lead and copper in acid solution are precipitated as sulphides by H_2S . [Pb, it should be remembered, is partially precipitated from strong solutions by HCl as chloride.] Copper is not precipitated by HCl or soluble chlorides.

The precipitated sulphides of Pb and Cu are insoluble in $(\text{NH}_4)_2\text{S}$ and KOH . Strongly acid solutions of these metals are not precipitated completely until suitably diluted with water. Lead sulphide (PbS), produced by adding H_2S water, or by passing H_2S gas, is black, insoluble in KOH , KCN , and $(\text{NH}_4)_2\text{S}$, but soluble in boiling dilute HNO_3 ; it is changed by boiling strong HNO_3 into white insoluble PbSO_4 .

Solutions of lead salts on addition of excess of dilute H_2SO_4 give white PbSO_4 .

K_2CrO_4 produces a yellow precipitate (PbCrO_4) soluble in KOH , insoluble in acetic acid.

KI precipitates yellow lead iodide (PbI_2), more insoluble in water than the chloride.

Quantitative Estimation. — Prepare a standard solution of $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, containing 0.0001 gramme Pb per c.c.

The molecular weight of lead acetate is 379, of which 207 parts are Pb. Accordingly $\frac{379}{207}$, or 1.831, grammes of the salt contain 1 gramme Pb. Dissolve therefore, with the aid of a little free acetic acid, one-tenth of this quantity—i.e., 0.1831 in a litre of

water—and each c.c. will contain 0.0001 Pb. To 100 c.c. of the water in a Nessler glass standing on a white tile add a few c.c. dilute acetic acid and sufficient H_2S solution to precipitate all the lead. To 100 c.c. of distilled water in a similar Nessler glass add the same amounts of acetic acid and H_2S solution, and run in from a burette or pipette the standard lead acetate solution until the depth of tint in the two Nessler's is exactly the same. Perform a second experiment, in which the whole volume of the standard lead acetate solution is added to the acidified distilled water at once, and then the H_2S solution added and well mixed.

The weight of Pb present in milligrammes per 100 c.c. (parts per 100,000) = 0.1 milligramme (0.0001 gramme) \times the number of c.c. of standard lead solution used.

It may be necessary to dilute the water sample, in which case a careful record of the amount of dilution must be made, and taken into account in calculating the result. Or it may be necessary to evaporate 500 c.c., or a litre, down to 100 c.c., and to use this for the estimation. Concentration may be necessary also in the qualitative examination.

Not more than 0.025 part Pb per 100,000 may be present in water without producing an effect when the water is drunk; 0.095 per 100,000 has proved fatal, and 0.050 is dangerous. In a word, all drinking water should be free from lead, as its poisoning action is increased through accumulation in the body.

Copper—Qualitative Examination.—There are two classes of copper salts—cupric and cuprous. Cupric salts are blue or bluish-green, and when freed from water of crystallization become pale or loss colour. Cuprous salts are usually white or colourless; they yield red Cu_2O when mixed with KOH , and white Cu_2I_2 when mixed with KI solution. CuO is black; Cu_2O red.

A little dilute NH_4OH added to solutions of copper salts produces a greenish-blue precipitate. More NH_4OH dissolves the precipitate, forming an intensely blue liquid.

KOH forms a pale blue precipitate, which when heated becomes black.

H_2SO_4 produces no precipitate; difference from lead.

$\text{K}_4\text{Fe}(\text{CN})_6$ produces a reddish-brown precipitate, $\text{Cu}_2\text{Fe}(\text{CN})_6$, insoluble in acetic acid.

H_2S throws down a brownish-black precipitate of CuS insoluble in KOH , $(\text{NH}_4)_2\text{S}$, in boiling dilute H_2SO_4 ; soluble in boiling HNO_3 and in KCN solution.

Quantitative Estimation.—Prepare a standard solution of copper sulphate containing 0.3929 gramme $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per litre. One c.c. of this solution = 0.0001 gramme Cu .

Dilute or concentrate the water if necessary, and place 100 c.c. in a Nessler glass as in the case of Pb . Add a few c.c. decinormal acetic acid and a few drops $\text{K}_4\text{Fe}(\text{CN})_6$ solution; a reddish-brown tint is produced. Match the intensity of this colour in a similar Nessler glass by mixing the necessary volume of standard copper solution with 100 c.c. distilled water and the same quantities of decinormal acetic acid and $\text{K}_4\text{Fe}(\text{CN})_6$ as were added to the glass containing the sample.

The number of c.c. of standard copper solution $\times 0.1$ gives the weight of Cu in the water in parts per 100,000, as in the case of Pb .

Not more than 0.1 part per 100,000 Cu is permissible in potable water.

Tin.—There are two classes of tin salts—stannous and stannic.

1. Pass H_2S into a solution of stannous salt acidified with HCl : a dark brown precipitate soluble in KOH and yellow ammonium sulphide forms on heating; reprecipitated by HCl from the KOH solution as brown SnS , and from the ammonium sulphide solution as yellow SnS_2 . [Note SnS is insoluble in colourless ammonium sulphide.]

2. Add HgCl_2 to acidified solution of a stannous salt: a white precipitate, Hg_2Cl_2 ; turns grey on boiling if the Sn salt is in excess through formation of metallic mercury and stannic chloride— $\text{Hg}_2\text{Cl}_2 + \text{SnCl}_2 = \text{Hg}_2 + \text{SnCl}_4$.

3. Add to the acidified stannous salt a drop of Br -water and a little AuCl_3 : purple precipitate. 'Purple of Cassius.'

Stannic salts in acidified solution:

1. H_2S : yellow precipitate of SnS_2 , soluble in both yellow and colourless ammonium sulphide; soluble in KOH on heating; reprecipitated by HCl as yellow SnS_2 from both solutions.

2. HgCl_2 : no precipitate.

3. AuCl_3 : no precipitate.

Quantitative Estimation of Tin (Stannous or Stannic).—In a measured quantity of water, concentrated if necessary to a small

bulk, precipitate the Sn as sulphide. Stand in a warm place till the smell of H_2S has nearly disappeared. Filter. Wash well. Dry. Ignite in the air into SnO_2 . Weigh, and calculate the Sn. [Incinerate the filter-paper apart from the precipitate, and add the ash to the crucible containing the SnO_2 .]

No tin should be present in drinking water.

Iron.—Ferruginous waters are found in mountain limestone, chalk, Bagshot sands, and greensands. They are generally opalescent, and slightly yellow in colour. The metal occurs as a bicarbonate which is readily converted into an insoluble carbonate, and also oxidized into the well-known 'rust'—hydrated ferric oxide, $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$. Ferrous salts decompose nitrates, absorbing O and producing nitrites, which in turn are further reduced to NH_3 . This reducing action accounts for the free NH_3 often found in pure waters derived from the greensands and other strata.

Chalybeate waters may be quite clear when drawn, but as oxidation of the Fe proceeds they become turbid and more or less brown. The insoluble and highly oxidized particles dissolve on the addition of a little dilute acid. Such turbidity may have its origin in iron pipes, cisterns, etc., in addition to strata.

Two classes of iron salts exist: ferrous, in which Fe is divalent, and ferric, in which it is trivalent. They may be readily distinguished by the three reagents, potassium ferrocyanide, $\text{K}_4\text{Fe}(\text{CN})_6$, potassium ferricyanide, $\text{K}_3\text{Fe}(\text{CN})_6$ (a solution always being made from the crystals immediately before use), and potassium sulpho-cyanide, KCNS .

| Reagent. | Ferrous Compound. | Ferric Compound. |
|--------------------------------------|---|--|
| $\text{K}_4\text{Fe}(\text{CN})_6$ - | Light blue precipitate, becoming dark blue on oxidation by the air, HNO_3 , or Br. | Dark Prussian blue, insoluble in HCl ; turned brown by KOH . |
| $\text{K}_3\text{Fe}(\text{CN})_6$ - | Dark blue precipitate; Turnbull's blue insoluble in HCl . | No precipitate. |
| KCNS - - | No red colour. | Blood-red colour (no precipitate). Colour destroyed by dropping a few drops into a solution of HgCl_2 . |

Sulphuretted hydrogen passed through a solution of a ferric salt reduces it to the ferrous state, with deposition of S. H_2S gives no precipitate with a ferrous salt in acid solution.

$(\text{NH}_4)_2\text{S}$ precipitates from a ferrous salt black ferrous sulphide. This reagent reduces a ferric salt to the ferrous state, and then precipitates ferrous sulphide with S.

Except in connection with greensands, ferrous iron is rarely met with in water work, ferric salts alone being found.

$(\text{NH}_4)_2\text{OH}$ produces with ferric salts a reddish-brown, flocculent precipitant, $\text{Fe}_2(\text{OH})_6$, insoluble in KOH, soluble in HCl.

$(\text{NH}_4)_2\text{S}$ precipitates black FeS soluble in HCl, insoluble in KOH.

These tests with the above reactions, produced by $\text{K}_4\text{Fe}(\text{CN})_6$ and KCNS are sufficient to identify ferric compounds in water.

Quantitative Estimation.—If the quantity of iron is small, it may be estimated colorimetrically like lead and copper, in which case prepare a standard solution of iron alum, $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, by dissolving 0.861 gramme in a litre of distilled water. This solution contains 0.0001 gramme Fe per c.c. If necessary, evaporate half a litre of the water to 100 c.c. Place this in a Nessler glass, and add 1 c.c. of dilute $\text{K}_4\text{Fe}(\text{CN})_6$ solution. Match the colour of the liquid in this glass by adding to 100 c.c. distilled water in a similar Nessler the same amount of $\text{K}_4\text{Fe}(\text{CN})_6$ and the requisite quantity of the standard iron solution. It is well to add a drop or two of nitric acid free from iron to each of the Nessler.

If the quantity of iron is too great for colorimetric estimation, acidify a litre of the water with HCl, and evaporate to dryness. Complete the drying in the air-bath at 150°C . Moisten with HCl, add water, and heat. Filter off any insoluble silica (which may be washed, ignited, and weighed). To the filtrate add a few drops pure HNO_3 , and boil. Then add a little $(\text{NH}_4)\text{Cl}$ solution and a slight excess of NH_4OH , and allow the precipitate of ferric hydroxide to settle. Filter this off; ignite and weigh as Fe_2O_3 .

[Should it be necessary to estimate Ca, which is now contained in the filtrate, add excess of ammonium oxalate, allow to settle, filter off, and ignite the calcium oxalate. Weigh as CaO .]

Not more than 0.1 part Fe per 100,000 should be present in a domestic water. A distinct chalybeate taste is produced by 0.3 part per 100,000.

Chromium.—Evaporate a litre of the water to be tested to dryness, and fuse the ash with solid potassium nitrate and sodium carbonate to produce yellow K_2CrO_4 , which, in neutral solution, produces a red precipitate with $AgNO_3$ (soluble in ammonia and dilute nitric acid), and in solution in acetic acid gives a yellow precipitate with lead acetate insoluble in dilute acetic acid. A few c.c. of a largely concentrated sample may be dropped on a thin layer of ether which has been floated on a dilute solution of H_2O_2 acidified with H_2SO_4 . Upon slight agitation the blue colour which forms in the lower solution passes to the ether.

In chromates (yellow or red in colour) Cr exists in combination with oxygen, acting as an acid radicle. Cr also forms a set of salts in which it acts as a metallic radicle. These are green or violet in colour, but pass through oxidation into chromates.

Conversely, chromates pass by reduction into green chromic compounds. Acidify a chromate with HCl, add Zn, and warm; the yellow chromate passes into a green chromic salt.

$(NH_4)OH$ and KOH in small quantity produce a pale bluish-green or purple precipitate of $Cr_2(OH)_6$, more or less soluble in excess of the precipitant.

Quantitative Estimation.—A chromate is first transformed by a reducing agent into a chromic salt. A solution of the chromic salt is then precipitated by NH_4OH in presence of NH_4Cl , and the resulting hydrate converted by ignition into Cr_2O_3 , and weighed. From the weight of Cr_2O_3 the amount of Cr is calculated.

No chromium should be present in a drinking water.

Zinc.—Concentrate the water.

$(NH_4)_2S$ produces a white, flocculent, gelatinous precipitate, which often appears yellow owing to excess of yellow ammonium polysulphide, $(NH_4)_2S_n$. This reaction is characteristic, as zinc sulphide is the only white sulphide capable of being precipitated. Zn is only partly precipitated from neutral solution by H_2S , but by adding sufficient $NaOH$, NH_4OH , or sodium acetate, the whole of the metal may be precipitated by this reagent.

Solution of NH_4OH gives a white precipitate of $Zn, (OH)_2$, readily soluble in excess of ammonia.

$K_4Fe(CN)_6$ produces a white gelatinous precipitate of zinc ferrocyanide.

Quantitative Estimation of Zn.—Prepare a standard solution of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. In 287 parts of this salt there are 65 of Zn, or in 4.4 parts 1 of Zn. Dissolve 4.4 grammes of the crystals in a litre of water (each c.c. = 0.001 gramme Zn). Use this standard solution volumetrically, as in the case of Fe, precipitating the Zn with $\text{K}_4\text{Fe}(\text{CN})_6$. Avoid much excess of the ferrocyanide. This may be effected by placing a drop of the mixture on a white tile in contact with a drop of a saturated solution of uranium acetate, when a brown colour appears immediately free ferrocyanide is present.

Gravimetric Estimation.—Heat a measured quantity of the concentrated water to boiling, and add slight excess of a solution of Na_2CO_3 . Boil again, and allow the precipitate to settle. Wash several times by decantation with boiling water; transfer the precipitate to a filter, and finish the washing thereon. When finished the wash-water shows no alkalinity to litmus and gives no precipitate with BaCl_2 .

Dry the precipitate, and carefully transfer it to a porcelain crucible. Heat to redness. Wet the filter-paper with strong ammonium nitrate solution, and dry it; incinerate it in the flame in a coil of platinum wire, and let the ashes fall into the crucible. The flame should not enter the interior of the crucible during ignition, lest reduction of the ZnO take place. Cool and weigh. Calculate Zn from ZnO .

Carbonate of Zn, ZnCO_3 , is found in certain mineral waters in quantities varying from 0.001 to 0.005 parts per 100,000. As much as 10 parts per 100,000 ZnSO_4 have been detected in such waters. Zinc may be introduced by galvanized iron tanks or pipes. It should not be found in a drinking water.

CHAPTER IV

ORGANIC MATTER IN WATER

As vegetable organic matter has little significance from the sanitary point of view, attention is almost entirely directed to animal matter in the form of sewage. It is not proved that animal organic matter *per se* in the quantities found even in dilute sewage is hurtful to health; its importance lies rather in the fact that pathogenic microbes, especially those of intestinal origin, accompany it. Wherever, then, faecal matters in quantity large or small are met with danger exists.

The complex remains of dead animals and plants are slowly changed to simple inorganic compounds in the superficial layers of the soil under the action of manifold ferments, the products of micro-organisms in association with favourable quantities of heat, moisture, and oxygen. The sum total of these changes is spoken of as an oxidation, since the end products are oxides of carbon, nitrogen, etc.; but there is no doubt that as in the case of the various fermentations which take place in the alimentary canal of animals, known collectively as digestion, reductions frequently alternate with oxidations. There is some evidence to show that these ferments, metabolic products of aerobic and anaerobic bacteria, act along certain lines which are intimately correlated. The specific action of one enzyme furnishes the necessary conditions for the opposed functions of a succeeding enzyme.

Whilst an accurate qualitative or quantitative estimation of organic matter in a potable water is impossible, still there are certain chemical tests of value in directing us towards the source of the organic matter, which source may ultimately be discovered by other means.

A rough differentiation of animal from vegetable matter may be

effected by a consideration of the ratio of 'organic carbon' to 'organic nitrogen,' which ratio forms the basis of Frankland's well-known method of estimating organic matter. The process is only suitable for experienced chemists and laboratories equipped with apparatus for gas analysis. But in skilled hands it is simple and direct. A measured volume of water is carefully evaporated to dryness; the residue is introduced into a hard glass tube along with some oxide of copper, and the tube is heated in a furnace until combustion of the organic matter is complete. The gaseous products of combustion—carbon dioxide, nitric oxide, and nitrogen—are severally collected and weighed, as 'organic carbon' and 'organic nitrogen.' If in surface waters the proportion of organic carbon to organic nitrogen be as low as 3 : 1 the organic matter may be considered as of animal origin, while if it be as high as 8 : 1 it is chiefly vegetable. In certain fresh peaty waters the ratio of C : N has been found as high as 12 : 1. In fresh sewage the proportion of C : N may be 2 : 1. Frankland held that the smaller the proportion of organic carbon and organic nitrogen in a water, and of these constituents the larger the proportion of C : N, other things being equal, the better is the quality of the water.

The fermentation of dead organic matter, known as 'putrefaction,' is effected by many types of micro-organisms.

Dead proteins are hydrolysed to proteoses; these to peptones; peptones to amino-acids; finally amino-acids are split, evolving ammonia.

If we follow this ammonia as it escapes, say, from a dung-heap in solution into the soil, we shall find that in the presence of the 'nitrous' organisms nitrous acid is formed, which in contact with the bases of the soil rapidly becomes nitrites. Later, through the activities of the 'nitric' group of micro-organisms, nitric acid is generated, which speedily becomes nitrates. These various stages in the oxidation or purification of nitrogenous matters stand out as chemical landmarks, and present considerable information to the water analyst.

As carbohydrates and fats are much less complex bodies containing C, H, and O only, their decomposition and oxidation are much more simple: carbon is burnt to CO_2 , and H to H_2O .

These changes in nitrogenous matter may be studied directly.

If, for example, A be a source of organic pollution, say a manure-heap, on the surface of the ground, and B, C, D, and E wells at increasing distances from it, analysis will show that the water in B contains abundance of NH_3 ; nitrification has not yet taken place. At C the oxidation processes have advanced to the stage of nitrous acid; this water will contain less ammonia and some nitrites. The water from D has travelled farther, encountering more nitrifying organisms, with the result that ammonia has disappeared, and nitrites and nitrates are found. At E purification is complete—the whole of the N is oxidized to nitric acid; hence this water contains no NH_3 , no nitrites, but only nitrates.

The opportunities for purification offered between A and B are not sufficient to carry the oxidation changes beyond the stage of NH_3 ; whereas the journey from A to E is of such length that the entire

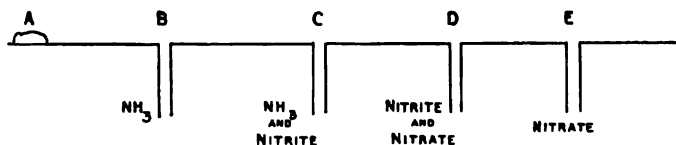


FIG. 3.

changes have been completed. At intermediate points are observed intermediate stages in the purification.

From the consideration of a single instance of this kind, no conclusions as to the distance a well must be removed from a source of contamination in order to be safe can be drawn, since the factors in the problem of safety are numerous and variable. The distance between A and E, if the water in E is to be completely purified, would require to be much greater if the slope from A to E be considerable, or E would need to be much deeper. On the other hand, if the slope of the ground water descended from E to A, it is possible that the water of B may be free from all organic matter. The porosity of the soil, conditions of heat and moisture necessary to vigorous growth of purifying organisms, direction of slope of ground water, geological features of subsoil and underlying strata, rainfall, and a number of other factors, all influence this question of safe distance of well waters from foci of contamination. Each case must be worked out on its own merits; and here the chemical

examination renders useful service. A sample of water from E. may be pure to-day—that is, contain no organic matter as such, no NH_3 , no nitrites, but only nitrates; to-morrow, owing to increased rainfall, whereby more organic matter than usual is washed into the soil, or to some other condition by which the powers of the soil for purification are lessened, this same water may contain, besides nitrates, nitrites, NH_3 , and even undecomposed organic matter. It should ever be borne in mind that the machinery by which organic matter is purified in the soil is liable at any point to break down, and in too many instances although just sufficient for the work is near breaking-point. The question, therefore, should be, not how near to a focus of contamination may it be safe to procure water, but rather how far from the focus is it possible to acquire it. Chemical analysis, if frequently and regularly performed, will, in most cases, discover such breakdown in the purification machinery, although a single analysis, unaccompanied by further information as to source and surroundings, may be quite useless. It is the comparative information regarding a water acquired by systematic and repeated analyses that is of value.

The student should note that the nearer the nitrogenous organic matter of domestic sewage stands to the stage of raw proteins the worse, as it is in this stage that pathogenic bacteria are found in their most toxic and vigorous condition; and that, conversely, the farther from this stage such matter stands the less dangerous it is. When organic matter reaches the stage of nitrates no pathogenic germs will live in it.

From the standpoint of infection, fresh faecal matter and urine are the most dangerous of all forms of organic matter.

It is not possible in water analysis to separate and estimate raw proteins, proteoses, peptones, and amino-acids. The next stage, that of NH_3 , lends itself to ready estimation.

When this 'free and saline' ammonia, as it is called, is removed, the remaining organic matter, which consists of the nitrogenous complexes constituting the antecedent stages, can be rapidly oxidized by the aid of a powerful oxidizer and heat (Wanklyn's process) into ammonia, and estimated as 'albuminoid' ammonia. This figure, inasmuch as it measures those portions of the nitrogenous organic matter likely to contain pathogenic micro-organisms,

is obviously the most important determination connected with this portion of the subject.

Estimation of 'Free and Saline' NH_3 .—Prepare a standard solution of $(\text{NH}_4)\text{Cl}$, 1 c.c. of which = 0.01 milligramme NH_3 .

53.5 grammes NH_4Cl contain 17 grammes NH_3 .
 3.14 " " " 1 gramme NH_3 .

Dissolve 3.14 dry anhydrous NH_4Cl in 1 litre ammonia-free distilled water. One c.c. of this solution = 1 milligramme NH_3 . This is too strong. Dilute 10 c.c. of it to a litre; 1 c.c. now = 0.01 milligramme NH_3 .

The process depends on the fact that when the water is distilled with a little sodium carbonate all the ammonia in the water, free or combined, passes over in the first portions of the distillate, and may be estimated by Nessler's solution.

Prepare Nessler's solution. Dissolve 62.5 grammes KI in about 250 c.c. distilled water. Set aside a few c.c. of this solution. Now add to the larger portion saturated mercuric chloride solution till precipitated mercuric iodide ceases to dissolve on stirring. Add the reserved KI so as to redissolve the precipitate, and again add cautiously sufficient mercuric chloride solution to produce a slight permanent precipitate.

Dissolve 150 grammes KOH in about 300 c.c. water; cool; add gradually to the above solution, and make up with H_2O to a litre. A brown precipitate settles out on standing, and the supernatant fluid is clear and of a pale greenish-yellow colour. It is ready for use as soon as it is perfectly clear. It should be decanted without stirring up the sediment. Keep in bottles closed with well-fitting rubber stoppers. This solution is rendered sensitive from time to time by the addition of a little more HgCl_2 solution; its sensitiveness depends on its being saturated with HgCl_2 .

Sodium Carbonate.—Heat anhydrous Na_2CO_3 to redness, taking care not to fuse it; transfer to a mortar, and grind to a fine powder. Store in a clean, dry, wide-mouthed, stoppered bottle.

Ammonia-free water is prepared by distilling ordinary water in the presence of Na_2CO_3 or H_2SO_4 , and rejecting the first portions of the distillate until there is no trace of colour produced on Nesslerising 50 c.c. of it.

A preliminary test may be made in order to ascertain what quantity of the water-sample should be distilled in order to make an exact determination of the ammonia. Place two Nessler glasses on a white tile; add 50 c.c. of the sample to one, and 50 c.c. ammonia-free distilled water to the other. To the ammonia-free water add 0.5 c.c. of the dilute standard NH_4Cl solution. To both Nessler's now add 2 c.c. Nessler's reagent, and stir. If on standing five minutes the intensity of colour in both cylinders is the same, 500 c.c. of the water may be used for distillation. If the intensity of the colour of the sample is much greater, dilution is necessary prior to distillation, otherwise the quantity of ammonia in the first 50 c.c. distillate will be too large to match.

Arrange a distilling-flask, condenser, and Bunsen burner. Pour into the flask 500 c.c. of the water (or water sufficiently diluted); add some prepared sodium carbonate, and if the water is acid a little more than usual (the least acidity fixes NH_3). Receive in Nessler glasses 150 c.c. distillate in three lots of 50 c.c. each. The boiling should be briskly effected; it is generally useful to place a piece of pumice in the flask to prevent bumping. As each Nessler glass is filled it should be Nesslerised or covered until Nesslerisation is accomplished.

Nesslerisation is one of a number of colorimetric methods of volumetric analysis in which the amount of a substance is estimated by adding to it a second body capable of forming a characteristic colour with it. The same conditions are accurately fulfilled in a similar vessel, using distilled water and such quantity of the substance sought, in standard solution, as will match the colour of the first when the same quantity of the second body is added. In order that slight differences in tint may be appreciated and matched, it is necessary to work with dilute solutions of the body to be estimated and the standard reagent; hence the necessity at times of diluting the water under examination.

Having collected the three 50 c.c.'s of distillate, Nesslerise each separately. Stand the Nessler glass on a white tile in a good north light, and by its side place a second Nessler glass of similar shape containing distilled ammonia-free water, and that quantity of standard solution of $(\text{NH}_4)\text{Cl}$ deemed necessary to match the first. Into each deliver 2 c.c. of Nessler's reagent, and carefully mix. In

a few minutes the yellow colour will have fully developed, and its depth can be gauged by looking down through the column. Should there be some discrepancy in the tints, rapidly add to another Nessler glass containing distilled ammonia-free water a little more or a little less of the standard solution, as the case may be, until an exact match is produced. In all such colorimetric work every condition should be exactly similar in the two cases—length of time reagents are in contact, order in which reagents are added, shape and size of containing vessels, etc. The standard solution of NH_4Cl must be added to the second Nessler glass before the Nessler's reagent, as this occurred in the Nessler glass containing the distillate. If the standard solution be added after the Nessler reagent an opacity is likely to form which prevents to some degree an exact match being made. Several trials may be necessary before an accurate result is reached.

The second and third 50 c.c. of the distillate are treated in the same way, and the sum of the results in terms of c.c. of the standard solution noted.

Wanklyn found that the whole of the free and saline NH_3 was contained in 150 c.c. distillate, and that the first 50 c.c. contained three-fourths of the total.

Nesslerise the second distillate first, and note whether more than 1.5 c.c. of the standard NH_4Cl solution is required to match it. If so, the first distillate must be diluted before Nesslerisation, otherwise the colour will be too intense to be accurately matched.

Example.

First Nessler glass matched by 3.00 c.c. NH_4Cl (1 c.c. = 0.01 milligramme NH_3)

Second " " " " 0.75 " " " "

Third " " " " 0.25 " " " "

Total $\text{NH}_3 = 4.00$ " " " "

But each c.c. standard $\text{NH}_4\text{Cl} = 0.01$ milligramme NH_3 ;

$\therefore 4 \text{ c.c.} = 0.04$ " "

And in 500 c.c. of the water under examination there is 0.04 milligramme NH_3 . In 100 c.c. there will be 0.008 milligramme NH_3 , or, since 100 c.c. water = 100,000 milligrammes, this water contains free and saline NH_3 to the extent of 0.008 part per 100,000.

Estimation of 'Albuminoid' NH_3 .—Whilst the Nesslerisation of the free and saline NH_3 is going on, 50 c.c. of alkaline potassium permanganate (composed of 200 grammes KOH, 8 grammes per-

manganate, a litre of water) should be boiled, so as to expel any ammonia that it may contain, and to heat the liquid in order to prevent cracking the retort when pouring it in. This is a strongly oxidizing reagent, and rapidly converts undecomposed organic matter into NH_3 . By this moist combustion process a degree of oxidation is effected in the course of half an hour or so in the laboratory that would require weeks or months by the natural processes outside.

When the alkaline permanganate is ready, the cork of the retort is removed and the hot solution poured in.

This portion of the distillation should be carried out more slowly, as organic matter is slowly decomposed, and the distillate should be collected as long as any NH_3 comes over. No relation exists between the number of the Nessler glasses collected and the total NH_3 , as in the case of the free and saline portion. Moreover, the second Nessler may contain as much NH_3 at times as the first.

The student should fit up his apparatus himself, and see that all connections are water-tight and gas-tight, as the case may be. Corks should be carefully bored and made to fit flasks and condenser tubes, and indiarubber corks are preferable to wood. The distilling-flask should be thoroughly cleansed with weak acid and rinsed out with distilled water until all traces of acid have disappeared. It is well to distil some pure ammonia-free water through the condenser in order to get rid of any traces of NH_3 that it may contain before starting the distillation of a sample. A large and constant stream of water running through the condenser is necessary throughout the entire process. A long-stem funnel is to be used for delivering water, etc., into the retort, and this is especially necessary for the introduction of the hot alkaline permanganate, so that none of the reagent may enter the central tube of the condenser and foul the distillate.

Seeing that the atmosphere of an ordinary chemical laboratory contains quantities of NH_3 , it is well to have a separate room for water analysis.

In very rare instances a potable water may not yield the entire free and saline NH_3 to the first 150 c.c. of the distillate. In such cases it will be necessary to distil over and Nesslerise a fourth or fifth 50 c.c.

It is possible that the 'saline' ammonia exists in water in conjunction with some acid, which, on being boiled in the presence of carbonates, yields up the ammonia in the form of $(\text{NH}_4)_2\text{CO}_3$.

In the second part of the process, the distillation of the albuminoid ammonia may require to be carried to a point at which the volume of fluid in the flask becomes dangerously small; this should never be allowed, but ammonia-free distilled water should be added to the flask as required, so that the volume may be kept up.

With regard to the amounts of 'free and saline' and 'albuminoid' ammonia which may be allowed in different potable waters, there is some little difference of opinion. All observers agree that the two ammonias must be considered together, and most agree that in drinking waters if the 'albuminoid' reach 0.005 part per 100,000 the 'free and saline' should not be more. If the 'albuminoid' be small—say less than 0.002 part per 100,000—the 'free and saline' may be allowed to slightly exceed 0.005.

Much 'albuminoid' and little 'free and saline' ammonia indicate vegetable matter; whereas much 'free and saline' and little 'albuminoid' indicate animal matter. These indications must not be too literally relied upon.

As a general rule, it may be stated that where a water has been contaminated with sewage the high 'free and saline' ammonia figure will be supplemented by an increase in chlorides, phosphates, and oxidized nitrogen. Whilst accepting the principle that animal pollution is indicated by a relatively larger figure for 'free and saline' ammonia than for 'albuminoid,' and that vegetable matter produces much 'albuminoid' ammonia, with little or no 'free,' it must be borne in mind that these relations are liable to be upset. Peaty waters, whilst producing 'albuminoid' ammonia in quantity, should not produce any 'free'; still, there are peaty waters met with at times which give rise to a small quantity of 'free' ammonia, although no animal matter can be traced.

Good spring waters rarely contain 'albuminoid' ammonia above 0.002 part per 100,000. Upland surface waters, as a whole, should not produce 'free and saline' ammonia beyond 0.001 part per 100,000.

The degree of initial dilution necessary to produce the best colour-tint for matching on Nesslerisation can only be discovered

by experience, and here, as in all matters practical, the student should ever appeal to experiment. Scores of waters must be patiently worked out in complete detail before he can expect to acquire even an elementary knowledge of the subject.

Much 'free and saline' ammonia in the absence of 'albuminoid' may be accounted for by the water passing through strata rich in ammonium salts, portions of which are carried away in solution; water-bearing strata containing nitrates and subsalts of iron afford 'free and saline' ammonia by the reduction of the nitrates through the intermediate phase of nitrous acid to NH_3 ; rain water falling through the atmospheres of towns abounding in ammoniacal fumes will yield appreciable quantities of 'free' ammonia, and at times small quantities of 'albuminoid' also from the organic matter in suspension in the air.

It may be noted that, although the Wanklyn process does not decompose urea, the most important and abundant nitrogenous constituent of urine, nor recover NH_3 from a few other bodies in sewage, still it is of the greatest value in dealing with the contamination of water by organic matter, from the comparative results afforded, so long as the determinations are carried out under similar conditions.

Oxidizable Organic Matter in Water.

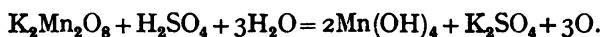
Forchammer applied to water analysis his knowledge of the experimental fact that organic matter in the presence of an acid can rob $\text{K}_2\text{Mn}_2\text{O}_8$ of a portion of its oxygen. This process was slightly modified by Tidy, and is usually known in this country in connection with his name. It is not a reliable test of either the quality or quantity of organic matter present, but, in that pure waters absorb practically no O from permanganates of potassium, and foul waters a great deal, the process has some value as corroborative evidence of the presence of organic matter. It should be noted that other bodies beside organic matter, such as ferrous salts, nitrites, sulphides, etc., abstract O from $\text{K}_2\text{Mn}_2\text{O}_8$, and when these are present they must be accounted for before drawing a conclusion as to the amount of organic matter dealt with. The quantity of O absorbed

varies with the time of contact, the temperature, and, to less extent, with the acidity, and light admitted during digestion.

Potassium permanganate in contact with organic matter and H_2SO_4 furnishes 5 atoms of O and colourless sulphates of manganese and potassium.



If sufficient acid be not added, the hydrated peroxide falls as an opaque brown precipitate, and only 3 atoms of O are set free.



During the digestion the reaction should be carefully watched, to see that the fluid remains transparent throughout. If much organic matter be present, it may be necessary to add further quantities of permanganate from time to time.

Various times and temperatures have been employed in this process for digesting the sample of water with the acid and permanganate, some analysts recommending four hours at $80^\circ \text{F}.$, others three hours, two hours, or fifteen minutes, at higher and lower temperatures. In a laboratory where an incubator is kept at blood-heat ($37^\circ \text{C}.$) it is convenient to use it, and three hours is a sufficient length of time. In examinations two hours at room-temperature may be found most convenient.

Prepare a standard solution of potassium permanganate (1 c.c. = 0.1 milligramme of available O) by dissolving 0.395 gramme of the pure crystal in a litre of distilled water. Make a fresh 10 per cent. solution of KI, and a fresh solution of sodium thiosulphate, of about 1 gramme to a litre of water. Lastly, prepare a boiled 1 per cent. solution of starch, and test its delicacy with water containing the merest trace of free iodine.

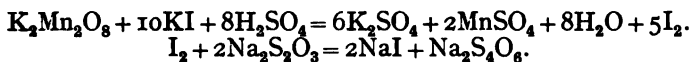
Clean two Erlenmeyer flasks (capacity 150 c.c. or less), and into one measure 100 c.c. of the water sample. Mark it 'Sample' with a wax pencil. Into the second, marked 'Control,' measure 100 c.c. distilled water. Now carefully pipette into each 10 c.c. of the standard solution of $\text{K}_2\text{Mn}_2\text{O}_8$, and with another pipette run into each 10 c.c. of a 25 per cent. solution of pure H_2SO_4 . Stopper and set aside in an air oven or incubator, as the case may be, at $37^\circ \text{C}.$ for a period of three hours. Should the amount of organic matter in the water be large, the whole of the permanganate may be de-

composed and become colourless; in such a case a second 10 c.c. of the standard solution is added, and should this be decolourized a third, and so on. Account of the further additions will be taken in the calculation at the end of the experiment.

When the time allowed has expired, and a portion of the permanganate remains undecomposed, as demonstrated by the red tint still to be seen, a few drops of the KI solution are added to the flask containing the water sample, when free iodine is liberated in quantity proportional to the amount of undecomposed $K_2Mn_2O_8$ remaining. A very few drops of the KI solution will contain an excess of iodine. This liberated I—the measurer of the undecomposed $K_2Mn_2O_8$ left in the Erlenmeyer—is made to oxidize thio-sulphate run into it from a burette, the end reaction being definitely ascertained in the presence of a few c.c. of the boiled starch solution by the disappearance of the blue colour of the iodide of starch.

The same procedure exactly is carried out with the control, and here, as no $K_2Mn_2O_8$ has been decomposed, but the whole of the 10 c.c. remains intact, we obtain a figure in terms of c.c. of thio-sulphate solution which represents this amount, or 1 milligramme available O.

The following equations represent the liberation of free I and its subsequent oxidation of sodium thiosulphate to sodium tetra-thionate:



Example.—The intact 10 c.c. standard solution of permanganate in distilled water liberated iodine equivalent to 27 c.c. of the thio-sulphate solution. The undecomposed portion of the 10 c.c. of standard permanganate in the water sample liberated iodine equivalent to 23.2 c.c. of thiosulphate. From this it is plain that the amount of permanganate solution decomposed by the organic matter (assuming that no nitrites, sulphides, etc., were present) is represented by 27 - 23.2 c.c. thiosulphate. But 10 c.c. standard permanganate or 1 milligramme O = 27 c.c. thiosulphate;

$$\therefore 27 : 27 - 23.2 :: 1 \text{ milligramme} : x;$$

$$x = \frac{(27 - 23.2) \times 1}{27} = 0.14.$$

There is, therefore, in 100 c.c. of this water organic matter capable of absorbing from permanganate of potassium O to the extent of 0.14 milligramme, or 0.14 part per 100,000, under the conditions of time and temperature employed.

If it be desired to obtain some indication of the nature of the reducing substances, two samples of the water may be treated with the standard permanganate, one at 37° C. for fifteen minutes, and the other for three hours at the same temperature. Nitrites, ferrous salts, and sulphuretted hydrogen effect reduction almost immediately, whilst a relatively large amount of ordinary organic matter reduces the reagent only after a considerable time.

The O absorbed from permanganate is higher as a rule in upland surface waters than in waters from other sources; and whilst no strict standards can be insisted on, it may be stated generally that in upland surface samples of great purity this figure in parts per 100,000 (time three hours, temperature 37° C.) will not exceed 0.1, in waters of medium purity 0.3, and in waters of doubtful purity 0.4. The corresponding figures for other sources will not exceed 0.05, 0.15, and 0.2

CHAPTER V

OXIDIZED NITROGEN—NITRITES AND NITRATES

DURING the early stages of putrefaction of organic matter much free N escapes in gaseous form, and the rest unites with H to form NH_3 . As has been already stated, certain bacteria in the soil and elsewhere convert NH_3 into HNO_2 , which latter combines with various bases to form nitrites. Of the so-called 'nitrous' organisms several species have been studied, one of which is the *Nitrosomonas* of Winogradsky. Nitrites, therefore, represent chemically the intermediate stage in the process of oxidation or purification. Under certain conditions, to be presently mentioned, they also represent an intermediate stage in the reduction of nitrates to NH_3 . The presence of nitrites in a water indicates more remote contamination in point of time or space, or of both, than does NH_3 .

In like manner 'nitric' organisms, such as the *Nitrobacter* (Winogradsky), transform HNO_2 into HNO_3 , which readily becomes nitrates. This class of bacteria has no action on NH_3 , and the previous class is unable to carry the oxidation of NH_3 further than HNO_2 , so that two distinct and independent types of organism are necessary to the complete oxidation of NH_3 .

It will be readily seen that the detection and estimation of nitrites and nitrates are of considerable importance in the investigation of the problem of organic pollution.

The presence of nitrates alone in a water indicates previous pollution that has been oxidized and rendered harmless. But if the quantity of nitrates be great, purified sewage may be suspected, which, through a breakdown at any moment in the machinery of purification, may become most dangerous sewage. Moreover, in view of the fact that sewage effluents contain almost as many micro-organisms as crude sewage, no effluent, however high its degree of

purification may be chemically, should ever be allowed to come in contact with drinking water. In all waters possessing a high nitrate figure this possibility of the presence of purified sewage should be borne in mind.

When nitrates, which form the end of the purification of organic matter, occur alone it is obvious that no indication of the date of the previous pollution is given.

In determining the true significance of nitrates in potable waters it is necessary to consider (1) whether they arise from geological strata (chalk, lias, oolite, sandstones) through which the water has percolated, in which case the evidence of organic pollution supplied by the other steps of the analysis—such as the 'free and saline' NH_3 , 'albuminoid' NH_3 , O absorbed from permanganate of potassium, etc.—will be negative; (2) whether they are due to purified sewage, in which case the quantity will be much too great, as also that of Cl; (3) whether they represent a small amount of organic matter that has undergone complete oxidation, and is to be considered harmless. In this case the quantity will be small—in rain and upland surface waters not exceeding 0.1 part per 100,000—and all the other items of the analysis employed to discover organic matter will afford negative evidence.

In the few cases where strata alone contribute soluble nitrates the quantity will rarely exceed 0.5 part per 100,000, but no figures can be laid down as an accurate standard, and each case must be worked out in connection with the rest of the analytical data.

In a few instances strata containing nitrates (in particular the lower greensand) contain also reducing minerals, such as proto-salts of iron, which reduce HNO_3 to HNO_2 , and the latter to NH_3 . The same reduction can be effected by denitrifying micro-organisms. Free NH_3 , due to reduction of nitrates and nitrites, will be identified by the absence of organic NH_3 , and all other evidence of organic pollution.

Nitrites are very unstable, and in the presence of available O rapidly become nitrates. In the early stages of the oxidation of large quantities of animal organic matter they are mostly found in company with NH_3 , but a foul water may at a particular moment fail to furnish any nitrites. They are significant of recent contamination, except in those cases just mentioned, where they are

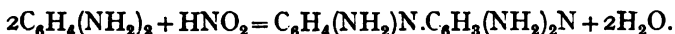
due to the reduction of nitrates in strata. Nitrites, then, which in deep-well waters may be merely the products of reduction of nitrates by iron in strata, iron pipes, etc., and consequently quite harmless, will in shallow wells and surface supplies condemn the water.

All the inorganic N—apart from strata—found in nitrates, nitrites, free and saline NH_3 , after deducting that present in rain water, may be regarded as due to previous sewage contamination.

Detection and Estimation of Nitrites.

Potassium Iodide and Starch.—To 10 c.c. of the water in a test-tube add 1 c.c. of a clear and boiled 1 per cent. starch solution and a drop of KI solution. Mix and add a little dilute H_2SO_4 , when immediately a blue colour is produced if nitrites be present in considerable amount. On standing, nitrates give this reaction also. Pure sulphuric acid should be used, and it is found that, owing to the instability of KI, ZnI gives better results. This test can be made a quantitative colorimetric one by operating on 100 c.c. of the water in a Nessler glass, and in a second Nessler 100 c.c. of a mixture of distilled water and the amount of a standard nitrite necessary to form a colour match. When the proportion of nitrites in a sample is 1 in 10,000,000, the blue colour is formed in a few minutes; when 1 in 100,000,000, in twelve hours; and when 1 in 1,000,000,000, in forty-eight hours. Lintner's soluble starch should be used.

Griess's Method.—Make a 5 per cent. solution of metaphenylene-diamine in water. Decolourize with animal charcoal, and render slightly acid with H_2SO_4 . Much acid must not be used. To 100 c.c. of the water to be tested in a Nessler glass add 1 c.c. of the reagent, cover, and set aside in a warm place for twenty minutes. A yellow to orange colour is produced, according to the quantity of nitrites present. The reagent should be made at the time of use. When metaphenylene-diamine (diamido-benzol) reacts with nitrous acid, triamido-azo-benzol (Bismark brown) is produced; hence the colour.



By using a standard solution of potassium nitrite, the colour produced in the 100 c.c. of water may be matched in the same

quantity of distilled water. A series of trials must be made, in which the reagent is added to the contents of the two cylinders at the same moment, and the cylinders covered and set aside in a warm place for twenty minutes. The standard nitrite is prepared thus: Dissolve 0.406 gramme of AgNO_2 in boiling water; add slight excess of KCl . Silver chloride is formed, and gradually falls to the bottom. Make up to a litre and allow to settle. When clear, decant off the supernatant fluid, and dilute each 100 c.c. up to a litre. It should be kept in the dark and in small bottles filled to the stopper, so as to protect it from the air.

1 c.c. = 0.01 milligramme N_2O_3 .

$$= 0.006 \quad \text{,,} \quad \text{NO}_2 = \left(\frac{\text{mol. wt. NO}_2}{\text{mol. wt. N}_2\text{O}_3} \times 0.01 \right).$$

$$= 0.0037 \quad \text{,,} \quad \text{N}_2 = \left(\frac{\text{mol. wt. N}_2}{\text{mol. wt. N}_2\text{O}_3} \times 0.01 \right).$$

Detection and Estimation of Nitrates.

Brucine Test.—To 10 c.c. of the water in a test-tube add 1 c.c. of a saturated solution of brucine, and shake. Incline the test-tube and pour down the side 2 c.c. of pure H_2SO_4 . Carefully bring the test-tube to the vertical against a white ground. A pink zone is formed at the junction of the acid and supernatant mixture, which lasts for a few seconds, and then changes to brownish yellow. When nitrates are in large quantity, the colour changes very rapidly. Where the reaction is doubtful, a fresh layer of the mixture can be brought in contact with the acid by imparting to the test-tube a slight centrifugal motion.

Or, 10 c.c. of the water may be evaporated to dryness in a platinum dish, a drop of pure H_2SO_4 added, and a small crystal of brucine dropped on the contents, when a pink colour will appear, even where the quantity of nitrates is so small as 0.01 part per 100,000.

Diphenylamine Test.—Mix about 10 milligrammes of diphenylamine with 1 c.c. pure H_2SO_4 in a porcelain basin, and carefully run $\frac{1}{2}$ c.c. of the water over the mixture. A blue colour develops in the presence of nitrates; the depth of the tint is roughly proportional to the amount of nitric acid. This reaction is not simulated by any other constituent of potable waters.

Crum's Quantitative Method.—This method consists in shaking up the residue obtained from the concentration of a measured quantity of the water with metallic mercury and pure H_2SO_4 , when nitric oxide is produced, which is afterwards conducted to a gas analysis apparatus and measured. It requires some experience in collecting and measuring gases, but in the hands of a skilled operator is one of the most exact methods known. The nitric oxide produced represents the N of nitrites and nitrates. To obtain the N due to nitrates alone, that obtained for nitrites by Griess's method is subtracted from the total. This method may be used for the estimation of nitrous and nitric N in sewage effluents.

Process of Estimation.—Evaporate to dryness in a dish 100 c.c. of the water. Add a small quantity 25 per cent. H_2SO_4 . Heat the dish to remove CO_2 from any carbonate present, and if the volume of the liquid exceeds 2 c.c. evaporate down to that volume. Fill the nitrometer with Hg, and pour the contents of the dish into the cup of the nitrometer, rinsing out with a very small quantity of the dilute H_2SO_4 . Now run the liquid through the stopcock, taking care that no air enters. Run through also about twice the volume of pure concentrated H_2SO_4 , and shake so as to cause part of the Hg to mix with the hot liquid. In a short time NO will be liberated. Continue the shaking till gas ceases to come off (five to ten minutes). Cool to the temperature of the air. Adjust mercury levels, and take the reading. Note atmospheric temperature and pressure, and calculate weight of N in volume of NO obtained.

An estimation gave 2 c.c. NO; temperature 18°C .; pressure 758 millimetres.

$$\frac{2 \times 273 \times 758}{291 \times 760} = 1.87 \text{ NO at N.T.P.}$$

As NO contains half its volume of N, and weight of 1 c.c. H = 0.000089 gramme, the weight of N in the NO = $\frac{1.87 \times 0.000089 \times 14}{2}$ = 0.001165 gramme = 1.165 part per 100,000 N in the water.

From this subtract the weight of nitrous N found by Griess's method; the remainder is that due to nitrates.

Copper-Zinc Couple Method.—This method estimates nitrous and nitric N as NH_3 . In calculating the nitric N, it is plain that from the amount of NH_3 obtained in the process deduction must

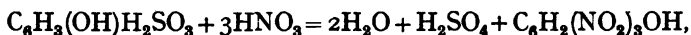
be made for original NH_3 in the water as well as that derived from nitrites.

When zinc is immersed in CuSO_4 solution, a spongy deposit of Cu is precipitated upon it, and in this condition it is capable of bringing about various decompositions in which H is liberated. The H is occluded by the spongy copper, and when thus occluded reduces nitrates to nitrites, and nitrites to ammonia. The reaction is hastened by the presence of traces of NaCl and other salts, rise of temperature, and any condition which increases the electrolytic action of the couple.

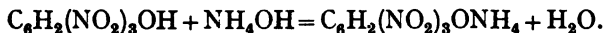
Take a piece of clean zinc-foil and cover it with 3 per cent. CuSO_4 solution until a copious, firmly-adherent coating of black spongy Cu has been deposited. This deposition should not be pushed too far, otherwise the Cu will be so easily detached that it cannot be washed. When sufficient deposit has accumulated, the CuSO_4 solution is removed and the couple carefully washed with distilled water, when it is ready for use. A clean, wide-mouthed, stoppered bottle is selected and washed out with some of the water to be tested. The coated foil is inserted, and a measured quantity—say 100 c.c.—of the water poured in so that the foil is completely covered. The bottle is tightly stoppered and set aside in a warm place for some hours. If the bottle be properly closed, the temperature may be raised to 28° or 30° C. without fear of losing NH_3 . When it is desirable to hasten the reaction, a little NaCl may be added to the water (0.1 gramme to 100 c.c.), or CO_2 may be passed through the water before it is placed in the bottle. In calcareous waters lime may be removed by the addition of some pure oxalic acid previous to digestion with the couple. Nitrous acid remains in the solution until the reaction is complete, so that it is necessary to test a small quantity of the water from time to time by Griess's reagent for the presence of this body. Metaphenylene-diamine easily detects 1 part of nitrous acid in 10,000,000 of water. When the last trace of nitrous acid has disappeared, the water is poured off the couple into a clean, stoppered bottle, and if turbid allowed to subside. A portion of the clear fluid, diluted if necessary according to the degree of concentration of the nitrates in the water, is transferred to a Nessler glass and the NH_3 estimated in the usual manner. In the case of coloured waters, or those containing magnesium and

other salts that interfere with the Nessler reagent, a measured quantity of the water poured off the couple should be put in a retort, a little Na_2CO_3 added, and Nesslerisation performed on the distillate. It has been found that about half a square decimetre of zinc-foil should be used for each 100 c.c. of a water containing 5 or less parts of nitric acid per 100,000. A larger proportion of foil should be used for waters richer in nitrates and for sewage effluents. The couple, if carefully washed after use, may be used for at least three estimations. It is convenient in most laboratories to digest overnight. Where accurate results are required, and in the hands of the inexperienced, it is advisable to distil the water removed from the couple and estimate the ammonia in the distillate. From the total N found as NH_3 deduct that due to inorganic NH_3 found by Wanklyn's process, and that due to nitrites found by Griess's process; the remainder is the N due to nitrates in the water.

Sprengel's Phenol Method.—This is a much less accurate method (error of under-estimation), but can be performed in a limited time. It estimates the N of nitrates alone, and is chiefly applicable to waters containing small quantities of nitric acid. When phenol sulphonic acid reacts with nitric acid, picric acid (trinitro-phenol) is formed.



and the ammonium salt of picric acid being yellow, this body lends itself to quantitative colorimetric estimation.



The solutions required are:

Standard potassium nitrate, containing 0.7215 gramme KNO_3 in a litre of water. One c.c. of this solution = 0.1 milligramme N. A dilution of 100 c.c. to a litre should be made for the analysis. One c.c. will then contain 0.01 milligramme nitric N.

Phenol Sulphonic Acid.—The phenol sulphonic acid used should be the pure disulphonic acid ($\text{C}_6\text{H}_3(\text{OH})\text{H}_2\text{SO}_3$), which, with HNO_3 , gives, according to Kekulé, picric acid even in the cold. Three grammes pure phenol and 37 grammes (20.1 c.c.) pure H_2SO_4 , specific gravity 1.84, are mixed in a beaker and heated for six hours

evaporation is made to take place rapidly in an open dish at 100°C . Slow evaporation at a lower temperature causes more loss, and the dry residues, if further heated, lose N. Chlorine does not interfere if present in less quantity than 2 parts per 100,000. If it exceed 7 parts, it should be removed before evaporation by Ag_2SO_4 . This process does not estimate the N as nitrite, as the action of nitrous acid results in the formation of nitroso-phenol, $\text{C}_6\text{H}_4(\text{NO})\text{OH}$, which is colourless in dilute solutions.

CHAPTER VI

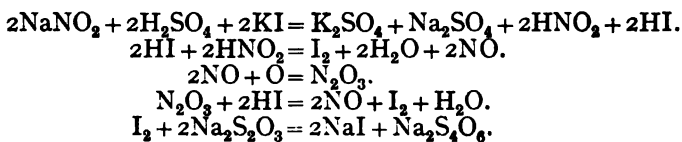
GASES IN WATER—WATER SEDIMENT—INTERPRETATION OF RESULTS OF CHEMICAL ANALYSES

WATER dissolves gases in quantities depending on temperature, pressure, and solubility of the gas. The principal gases found in potable waters are N, O, CO₂, and occasionally CH₄, H₂S, and NH₃. Of these O and CO₂ are alone worthy of estimation. As organic matter in water throughout all its stages of change lays hold of dissolved oxygen, the presence or absence of this gas may afford valuable information regarding such organic material. These remarks apply equally to sewage effluents.

From a hygienic point of view the subject of gas extraction from waters is not sufficiently important to warrant the expenditure of time and labour inseparable from accurate gasometric work. Nor is the information gained, even when the work is most exactly performed, of constant or certain value.

Estimation of O Dissolved in Water (Thresh).

When sulphuric acid is added to a mixture of KI and a nitrite, iodine is set free. If O be carefully excluded, this free iodine rapidly reaches its maximum, and remains constant. But if O be admitted, the amount of iodine liberated varies with the time of exposure, and has no relation to the amount of nitrite present. Thresh concludes that the NO produced acts as a carrier of O, forming N₂O₃, which liberates more iodine and is again transformed into NO, and that this action continues as long as any free dissolved O remains in the water.



In the above reactions it will be noted that free nitrous acid is first formed, and that this liberates I.

If now the total amount of I liberated be determined, and the

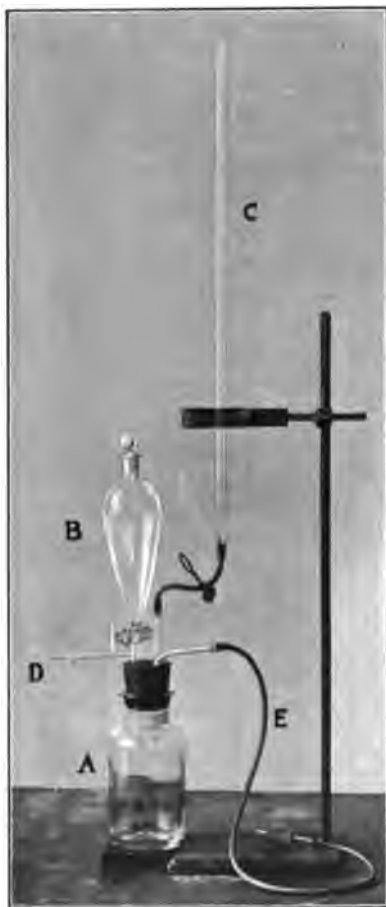


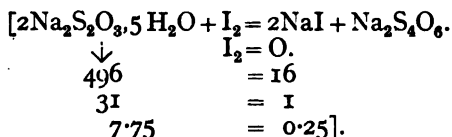
FIG. 4.

amount of I theoretically liberated by the nitrite be calculated, the difference will represent the I liberated by the O dissolved in the water.

The estimation is carried out as follows (see Fig. 4):

Into a wide-mouthed glass bottle A, of 500 c.c. capacity, is fitted an indiarubber cork, with four perforations. The stem of a separator funnel B, holding about 300 c.c. of the water, is pushed through the cork. Through another perforation is run a piece of glass tube attached by rubber tubing to the lower end of a 100 c.c. burette C, graduated to tenths of a c.c. Through the remaining two perforations are run pieces of glass tubing bent at right angles, one D, connected by rubber with a gas-tap, and the other E, by similar tubing with a short piece of glass tube thrust through an indiarubber cork, which fits the top of the separator funnel. The funnel B is filled with water to the top, and the glass stopper inserted, displacing a small quantity of water. The contents are accurately measured once for all, and the capacity of the funnel noted.

The funnel is now filled with the water to be examined. The burette C is charged with thiosulphate (1 c.c. = 0.25 milligramme O), made by dissolving 7.75 grammes of crystalline sodium thiosulphate in a litre of distilled water.



Having thoroughly cleaned and dried the bottle A, the cork is inserted and the tube connected with the lower end of the burette C, fixed in position. The funnel B is filled up to the top, and the stopper inserted; the stopper is now taken out and 1 c.c. of a solution of sodium nitrite and potassium iodide (sodium nitrite 0.5 gramme, KI 20 grammes, distilled H_2O 100 c.c.) poured in from a 1 c.c. pipette. From a second 1 c.c. pipette is run in 1 c.c. H_2SO_4 (25 per cent.). The higher specific gravity of the nitrite mixture and of the H_2SO_4 solution causes these to sink rapidly to the bottom of B, and when the stopper is replaced a negligible quantity, if any, of the reagents just added is lost in the small amount of water which overflows; in this way the entry of air is excluded. The funnel is inverted a few times, so as to effect a uniform admixture, and its nozzle pushed through the cork. The tube D is joined up with a gas-tap, and gas rapidly passed through the bottle. When

all the air has been expelled the gas may be lighted at the end of E, where it will burn quietly. The stopper of B is removed, and having rapidly extinguished the flame at the end of E, the cork of the latter is fixed in B, after which the tap is turned, and the mixture of water and nitrite solutions is discharged into A. The tap of B is now turned off, the cork at the end of E removed, and the gas relighted and turned down to a small flame. Thiosulphate is then run in slowly from C until the brown colour produced by the liberated iodine is nearly removed. About 3 c.c. of a fresh starch solution is poured into B, and 1 c.c. of this carefully run through the tap into A, in order to definitely fix the end of the reaction. As the blue colour returns in most instances after a few seconds, it is well to wait for a little and add a further drop or two of thiosulphate to complete the decolorization.

The amount of thiosulphate used will represent:

- (1) The I (and accordingly its equivalent as O) liberated by the nitrite in the reagent.
- (2) The I (and its equivalent O) liberated by the nitrite, if any, originally in the water.
- (3) The O dissolved in the reagents.
- (4) The O dissolved in the water sample.

The value of (4) can obviously be determined by subtracting the sum of the values (1), (2), and (3) from the total.

The values of (1) and (3) can be easily determined by making a blank experiment, using five times the amounts of nitrite-iodide solution, sulphuric acid, and distilled water in lieu of thiosulphate, as it may be assumed that the oxygen in distilled water is equal to that in thiosulphate. The number of c.c. of thiosulphate solution required divided by 5 gives the joint values of (1) and (3). In order to estimate (2) the nitrous acid in the sample must be very carefully determined, and as 94 parts by weight of this are equivalent to 16 of O, the calculation is easily made [$2\text{HNO}_2 \rightarrow \text{O}$].

For a given piece of apparatus, the values of (1) and (3) having been once determined, it is unnecessary to repeat the process, granted that the same quantities of reagents are always used. In (2) the nitrous acid may be estimated by Griess's method.

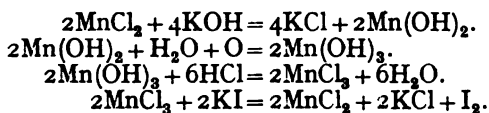
A simpler method for the estimation of O dissolved in water is that of **Winkler**:

In this method manganous hydrate serves as the oxygen carrier, and enables it to liberate its equivalent of iodine, which is then titrated in the usual way.

In collecting the sample of water, care must be taken to avoid agitating it and exposing it for any length of time to the air. It is transferred with similar precautions by syphoning to a stoppered bottle of known capacity—say 250 c.c. One c.c. of strong manganous chloride solution (40 grammes $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ to 100 c.c.) and 2 c.c. of a solution containing 33 per cent. KOH and 10 per cent. KI are introduced by a pipette with long stem which carries its contents to the bottom, thus displacing 3 c.c. of water from the top. The bottle, which must be full of liquid, is now closed with the stopper without including any air-bubble, and the liquids are mixed by several times inverting the bottle. The manganous hydroxide precipitate which forms will be more or less discoloured by higher hydroxide, according to the proportion of O which was dissolved in the water sample. As the oxidation of the manganous hydroxide is not immediate, and the result is influenced by light, the bottle is put aside in a dark cupboard for fifteen minutes; 5 c.c. strong HCl are added, which cause the precipitate to disappear, and leaves the liquid coloured with dissolved iodine. The iodine is titrated with standard thiosulphate, of which the oxygen value should be known, so as to give the amount of oxygen directly. If 250 c.c. of water be used, it will be convenient to use a solution of thiosulphate of 7.75 grammes to the litre [1 c.c. = 0.25 milligramme O], as then each c.c. thus used can be read as 1 milligramme O dissolved per litre of water. It is usual, however, to determine the amount of thiosulphate required by the same volume of fully aerated pure water of similar character, or of distilled water, and then to calculate the percentage of the possible amount of oxygen present in the polluted water directly from the amounts of thiosulphate which equal volumes of the two samples require. The manganous chloride must be free from iron, and all the reagents must be free from nitrites.

It has been objected that iodometric methods are inapplicable to waters containing much organic matter, as this may absorb iodine—but this objection does not appear to be well founded.

Ordinary tap water at room temperature contains about 7 c.c. O dissolved per litre, or by weight about 1 part per 100,000.



CO₂ in Water.

Carbon dioxide may exist in solution in water in the free state, as a bicarbonate, or as a carbonate.

Estimation of Total CO₂, (free CO₂, CO₂ in bicarbonates, CO₂ in carbonates).—Solutions and apparatus required:

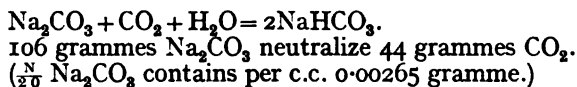
BaCl₂ solution, 10 per cent. H₂SO₄, baryta-water, flask of capacity about 300 c.c., fitted with a perforated bung through which three holes are bored, the first carrying a funnel tube provided with a stopcock, to hold H₂SO₄; the second carrying a glass tube almost to the bottom of the flask, and connected outside to a bottle containing baryta-water; and the third carrying a glass tube connected with a CaCl₂ tube and a weighed potash bulb containing 50 per cent. solution of KOH.

Process.—Measure into the flask 200 c.c. of the water to be examined, and 50 c.c. baryta-water, together with 5 c.c. BaCl₂. Shake, and allow to settle for twenty-four hours. Decant off as much of the clear fluid as possible without disturbing the sediment. Should there be a scum on the surface, rapidly run the fluid through a filter-paper, and drop the filter into the flask. Replace the bung and run in slowly the H₂SO₄, which decomposes the carbonates. The Ba(OH)₂ has previously precipitated as carbonates all the free CO₂ and that existing as bicarbonate. The total CO₂ evolved by the action of H₂SO₄ is absorbed by the KOH in the bulb. Weigh the bulb, and difference in weight represents this CO₂.

When during the experiment the CO₂ ceases to come off, the flask should be gently heated in order to assist the evolution, and air drawn through in order that all the CO₂ may reach the KOH.

Estimation of Free CO₂.—Measure into a porcelain basin 100 c.c. of the water; add a few drops of phenolphthalein, and run in from a burette a solution of $\frac{N}{20}$ Na₂CO₃ until a faint red colour is

developed. The sodium carbonate forms with the CO_2 sodium bicarbonate (NaHCO_3), and immediately all the CO_2 is used up further carbonate turns the indicator red. The amount of Na_2CO_3 used measures the quantity of CO_2 present.



1 c.c. of the sodium carbonate therefore neutralizes $\frac{44}{106} \times 0.00265$ gramme $\text{CO}_2 = 0.0011$ gramme CO_2 .

If in an estimation it is found that 3 c.c. $\frac{N}{20}$ Na_2CO_3 are required to neutralize the CO_2 in 100 c.c. water, the amount of CO_2 in this water will be 3×0.0011 gramme = 0.003 gramme = 3 milligrammes = 3 parts per 100,000.

Estimation of Free CO_2 and CO_2 as Bicarbonate.—If to 100 c.c. of the water a little BaCl_2 be added to precipitate carbonates, sulphates, and phosphates of any alkalis which might be present, and which would precipitate barium from baryta-water; and, further, if a little saturated ammonium chloride be added to prevent the precipitation of magnesia (MgCO_3 would precipitate BaCO_3 from $\text{Ba}(\text{OH})_2$), the CO_2 existing free and as bicarbonate may be neutralized by excess of $\text{Ba}(\text{OH})_2$; and the loss in alkalinity of the measured excess of $\text{Ba}(\text{OH})_2$ solution used may be estimated by titration with standard oxalic acid (as carried out in Pettenkofer's method of estimating CO_2 in the air, *cf.* p. 133), and converted into terms of CO_2 .

The CO_2 due to bicarbonates is equal to the figure found for this estimation less that for free CO_2 .

Estimation of CO_2 as Carbonates and Bicarbonates.—To 100 c.c. of the water add a few drops of phenolphthalein, which immediately becomes red from the action of the carbonates (phenolphthalein remains colourless in the presence of bicarbonates). Run in standard oxalic acid, 1 c.c. = 1 milligramme CO_2 , until the indicator loses colour. This measures the carbonates.

Now boil the water for fifteen minutes, and run in further standard oxalic acid until the phenolphthalein, which in the meantime has become coloured, again becomes colourless.

The first addition of acid converts the carbonates into bicarbonates: hence colourless phenolphthalein.

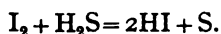
Boiling converts the bicarbonates (original and converted) into carbonates: hence red phenolphthalein.

The second addition of acid measures the CO_2 after boiling. Twice this figure represents the CO_2 as bicarbonates (original and converted) before boiling. If from this last figure be subtracted the first figure found—viz., the CO_2 as carbonates—the remainder is the amount of CO_2 as original bicarbonates.

One hundred c.c. of a water required 3 c.c. oxalic acid, which means that it contains 3 parts CO_2 as carbonates, per 100,000. When boiled and titrated further 4 c.c. of acid were required. Total bicarbonates therefore equal $4 \times 2 = 8$ milligrammes. Lastly, $8 - 3 = 5$; therefore the original bicarbonates in the water amounted to 5 parts per 100,000.

Or these estimations may be carried out without heat by titrating with a standard acid and two indicators—phenolphthalein and methyl orange. When the phenolphthalein has become colourless (end of carbonates estimation) methyl orange is added, and addition of acid continued until the indicator proclaims the presence of free acid (end of bicarbonates estimation). Methyl orange is sensitive to bicarbonates.

Sulphuretted hydrogen in water may be estimated by titrating a measured quantity with $\frac{N}{80}$ I.



A drop or two of boiled starch solution is used to fix the end-point.

$$[1 \text{ c.c. } \frac{N}{80} \text{ I} = 0.34 \text{ milligramme } \text{H}_2\text{S}.]$$

WATER SEDIMENT.

The biological examination of a water sediment may throw much light on the problem of its origin and the nature and mode of its contamination. A $\frac{3}{8}$ -inch and $\frac{1}{8}$ -inch objective of the ordinary English microscopes furnish good fields for this work. The number of possible organic forms—animal and vegetable—that may contaminate a water is so great that no expert could be expected to recognise all. But in the search for sewage pollution a number of unmistakable objects may be seen that will clinch the diagnosis. The micro-chemical examination of mineral particles, such as iron

compounds, carbonates, oxalates, etc., is in certain cases of some import; but the investigation of animal and vegetable matter is much more likely to lead to positive evidence of sewage and other organic forms of pollution.

In this chapter a few, and only a few, general remarks will be made on the biological examination, and the student will do well to consult such works as Cooke's 'British Desmids,' 'Fresh-water Algæ,' by the same author, Whipple's 'Microscopy of Drinking Water,' and other writers on the Infusoria, Rotifera, Fungi, etc. A necessarily limited number of illustrations are given, but it is hoped that these will be sufficient to introduce the beginner to the microscopic study of water sediments, which in every examination should be faithfully carried out.

Much has been written on methods of procuring the sediment. Where a centrifugal machine is at hand it is most satisfactory to use it, and where none can be had the ordinary conical urine glass suffices in every respect. In using the latter, the water should stand overnight. The clear fluid is carefully syphoned or poured away, and the sediment at the bottom is removed by a fine pipette, and dropped in single drops on a series of microscopic slides. Some workers use well-slides. Should there be a scum on the surface of the water in the conical glass, this is removed separately and transferred in like manner to slides. Cover-glasses are applied, and the slides carefully examined, first by the low and afterwards by the higher objective.

Certain biological forms inhabit only foul water, and disappear when it becomes purified. Where a supply usually satisfactory develops colour, turbidity, or odour, a microscopical examination alone may elucidate the causes. A satisfactory water should be free from all suspended matter, and especially from all living and dead animal and vegetable matter. Certain animal and vegetable growths may occur in storage reservoirs and cisterns through the admission of light to the water: plants containing chlorophyll (green algæ, diatoms, etc.) grow in light. The different seasons bear different forms and amounts of animal and vegetable life, therefore a systematic microscopical examination is necessary. Vegetable growths may take place at dead ends in mains. Much dead organic matter will be found in the form of unrecognisable débris, but

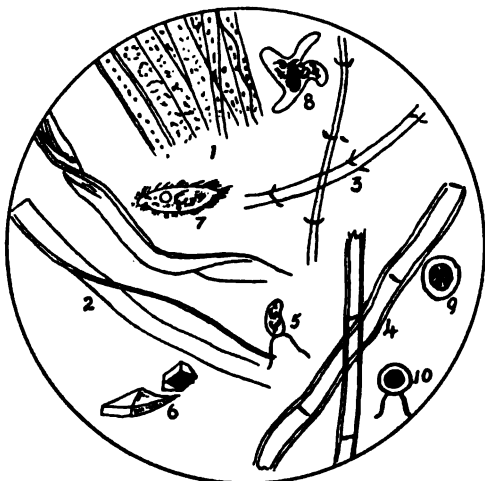


FIG. 5.

- | | |
|--------------------|------------------------|
| 1. Wood cells. | 6. Particles of sand. |
| 2. Cotton fibre. | 7. Paramecium. |
| 3. Linen fibre. | 8. Amoeba. |
| 4. Hemp fibre. | 9. Encysted Infusorian |
| 5. Algal zoospore. | 10. Algal zoospore. |

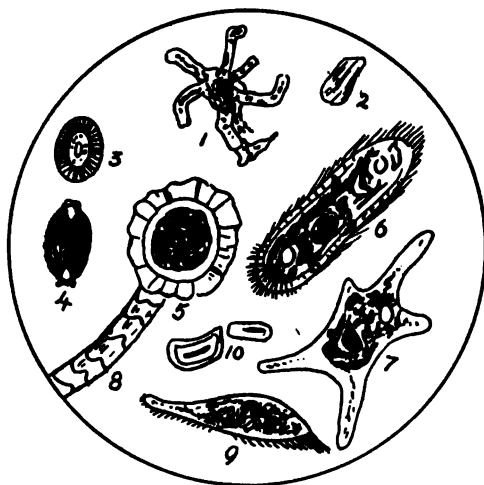


FIG. 6.

- | | |
|--|-----------------------------|
| 1. Fresh-water Hydra. | 6. Paramecium. |
| 2. Scale of insect. | 7. Amoeba. |
| 3. Egg of <i>Tænia solium</i> . | 8. Wool fibre. |
| 4. Egg of <i>Trichocephalus dispar</i> . | 9. <i>Euplotes Charon</i> . |
| 5. Egg of <i>Ascarus lumbricoides</i> . | 10. Diatoms. |

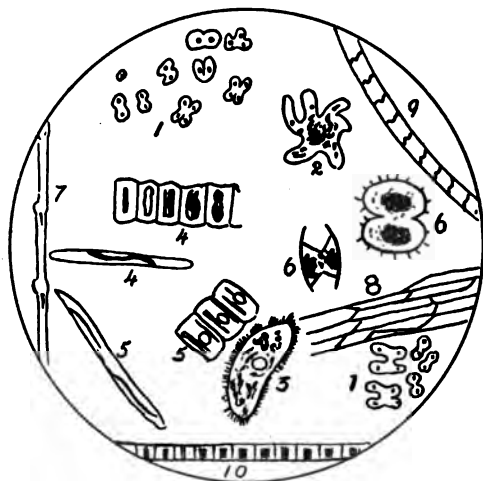


FIG. 7.

- | | |
|--------------------------|----------------------|
| 1. Pleurococcus (Algæ). | 7. Hair of insect. |
| 2. Amœba (Protozoa). | 8. Vegetable tissue. |
| 3. An Infusorian. | 9. Fibre of wool. |
| 4 and 5. Diatoms (Algæ). | 10. Ulothrix (Algæ) |
| 6. A Desmid (Algæ). | |



FIG. 8.

- | | |
|----------------------------|--------------------------|
| 1. Anguillulæ (Nematoda). | 6. Chara fragilis. |
| 2. Ulothrix. | 7. Diatom (Synedra). |
| 3. Zoogloea of micrococci. | 8. Uroglena. |
| 4. Anabena. | 9. A Desmid (Cosmarium). |
| 5. Cryptomonas. | 10. Encysted Infusorian. |



FIG. 9.

- | | |
|-------------------------|--------------------------|
| 1. Vorticella. | 6. Crenothrix polyspora. |
| 2. Spirogeira. | 7. Volvox globator. |
| 3. Sphaerotilus natans. | 8. Tabellaria. |
| 4. Beggiatoa. | 9. Species of Nostoc |
| 5. Daphnia. | 10. Melosira. |

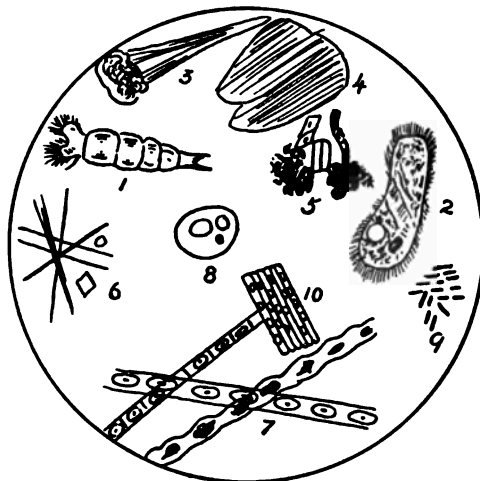


FIG. 10.

- | | |
|-----------------------------|----------------------------------|
| 1. Rotifer (Annuloida). | 6. Crystals of calcium sulphate. |
| 2. Paramecium (Protozoa). | 7. Algal filaments. |
| 3. Animal spine. | 8. Not identified. |
| 4. Wing scale of an insect. | 9. Bacteria. |
| 5. Vegetable debris. | 10. Diatom. |

amongst it much that is recognisable, as epithelium, striped muscle, cotton, silk, and linen fibres, starch granules, dotted vegetable ducts, wool, hair, ova of intestinal worms, and numerous other bodies, all distinctive of sewage. It will thus be seen that a knowledge of the fauna and flora of water will enable workers to recognise certain organisms, alive or dead, which produce odours in water, others which live only in pure waters, and whose presence excludes gross pollution, and those which live in polluted waters, and consequently point to sewage or other contamination. Fishy odours, according

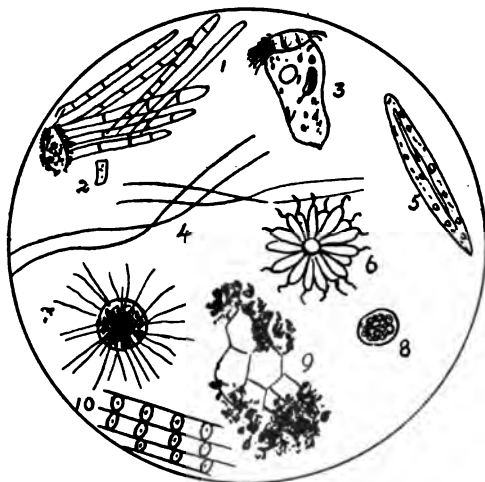


FIG. II.

- | | |
|--|--------------------------------------|
| 1. Oscillatoria. | 7. A Heliozoon. |
| 2. Small Infusorian. | 8. Egg of an Entozoon. |
| 3. Free-swimming Vorticella. | 9. Pith cells partially covered with |
| 4. Cotton fibres. | vegetable debris. |
| 5. Navicula (Diatom). | 10. Wood of a Conifer. |
| 6. Confervoid Alga (<i>Synura uvella</i>). | |

to Whipple, are produced by *Endorina*, *Volvox*, *Pandorina*, and other *Chlorophyceæ*, *Uroglena*, *Bursaria*, and other *Protozoa*. Aromatic odours are created by numerous diatoms—*Tabellaria*, *Meridion*, *Diatoma*, etc.—and *Protozoa*. Grassy odours are produced by *Rivularia*, *Anabæna*, *Cælosphærium*, and other *Cyano-phyceæ*.

In river water and unfiltered supplies possessing odours the organisms are likely to be found in the supply; whilst in filtered waters they mostly grow on the filters. The foul odour and reddish colour of the Cheltenham water some years ago was shown to be due to a species of *Crenothrix* growing in the reservoirs and on the filters. In deep-well and spring waters any low forms, animal or vegetable, indicate insufficient protection from light, such as storage in uncovered reservoirs. The so-called sewage fungus, *Beggiatoa* alba, including *Carchesium* *Lachmanni*, and other forms, occurs in

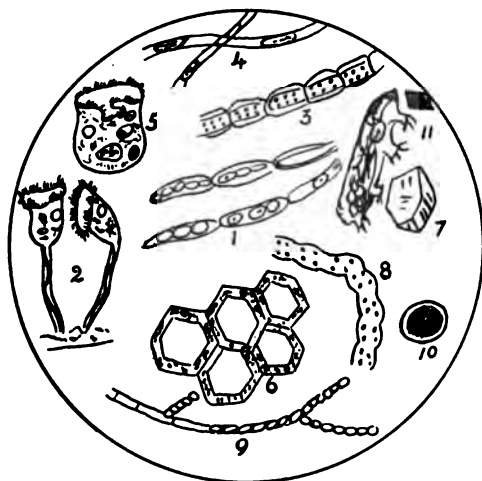


FIG. 12.

- | | |
|--|---|
| 1. <i>Leptomitrus lacteus</i> (from impure river). | 6. <i>Hydrodictyon</i> (fresh-water Alga) |
| 2. <i>Carchesium</i> <i>Lachmanni</i> (from water polluted with sewage). | 7. Sand particles. |
| 3. <i>Conferva bombycina</i> (pond water). | 8. Algal filament. |
| 4. Fresh-water Alga (<i>Lyngbya</i>). | 9. Hypha of fungus (sporing). |
| 5. <i>Bursaria gastris</i> . | 10. Encysted Protozoon. |
| | 11. Water bear. |

effluents from sewage-farms and bacteria-beds. *Beggiatoa* also occurs in river beds and stagnant waters containing H_2S . Wino-gradsky holds that it does not produce the S which it contains in the dried state, but that this S is derived from the H_2S by other means. Cohn states that it produces S from sulphates and albuminous bodies.

The organisms forming the slimy superficial layer (*Schlammdecke*)

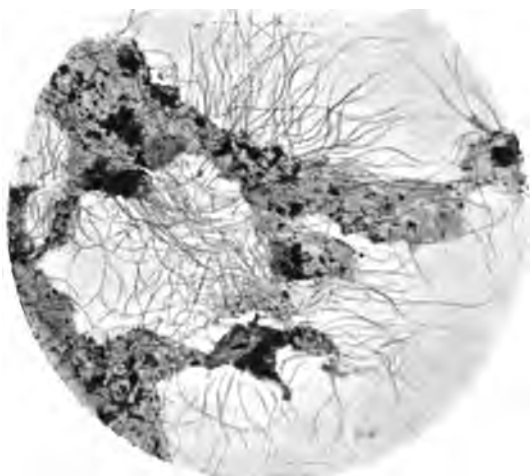


FIG. 13.—*Beggiatoa alba*.



FIG. 14.—*Daphnia pulex*.

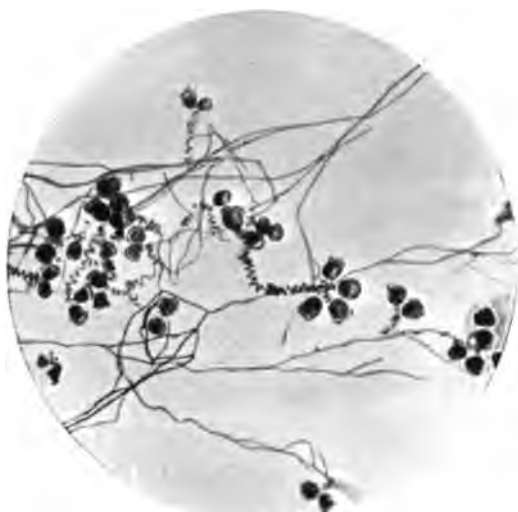
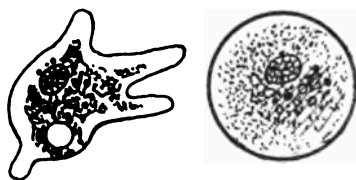


FIG. 15.—VORTICELLA.



Motile.

Resting.

FIG. 16.—AMOEBA COLI.



FIG. 17.—LEICESTERSHIRE WOOL.

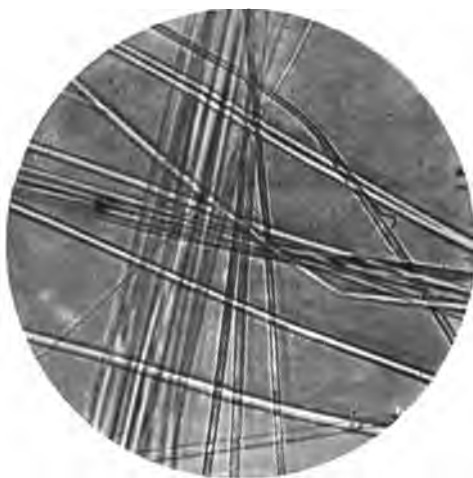


FIG. 18.—CHINESE SILK.



FIG. 19.—FLAX FIBRES.



FIG. 20.—HEMP FIBRES.



FIG. 21.—JUTE FIBRES.



FIG. 22.—COTTON FIBRES.

of a sand-filter are innumerable, and vary with the source of the water and other factors.

When a sand-filter is first set to work, it acts merely as a strainer. In the course of a few days a slimy organic layer consisting of green and blue algæ, fungi, zoogloea masses of bacteria, diatoms, and a multitude of other organisms, makes its appearance, and true filtration then commences. The source of the water, season of the year, etc., determine the presence of specific forms. Certain green algæ are produced in the spring, blue algæ in the summer, and their colouring matter may be liberated at any time and remain on the surface long after the organisms have died.

The matter obtained as sediment from a centrifugal machine, conical glass, or surface scum, when examined microscopically, may be found to contain (1) living animal forms, (2) dead animal forms, (3) living vegetable forms, (4) dead vegetable forms, (5) mineral detritus, and (6) unrecognisable débris, requiring micro-chemical and other methods of investigation.

The differentiation of some lowly animal and vegetable organisms is frequently a matter of no little difficulty, but careful search should be made for these, as their presence has special significance.

INTERPRETATION OF RESULTS OF CHEMICAL ANALYSES.

As previously indicated, judgment should be exercised at all times in expressing an opinion on a water without a personal inspection of the source, etc.; but many instances will arise in which no doubt can exist as to the foulness of the sample. Positive results in the search for sewage contamination are much more easily dealt with than negative. The liability to such pollution should ever be kept before the mind of the analyst. Deep springs and wells for the most part afford the purest waters. Upland surface waters may be also quite pure. But subsoil waters and waters from cultivated lands, as also most river waters, are rarely free from pollution. Waters collected from the surfaces of the more impervious rocks destitute of animal and vegetable life, are extremely pure. These rarely contain any appreciable NH_3 , and rarely more than 1 part chlorine, 0.1 part nitric N, 5 parts hardness, and 10 parts total

solids per 100,000. Waters collected from rocks covered with peat will present high figures for organic ammonia, and O absorbed by organic matter, and their acidity will be great. Such waters are plumbo-solvent, and should be neutralized before distribution to the consumer. Waters from mountain limestone are moderately hard, with high total solids and neutral or faint alkaline reaction. The mineral residue is chiefly composed of carbonate and sulphate of calcium and magnesium. Great variety in composition is found amongst waters originating in the lias, magnesium limestone, red sandstone, and oolite; total solids may range from 10 to 15 parts; total hardness 10 to 15; chlorine 1 to 2; and nitric N 0.1 to 0.2. Alluvial strata furnish waters of high total solids (50 to 100); and waters from cultivated soils vary within very wide limits in total solids, hardness, chlorine, and nitric N.

Hard waters are derived from the chalk, limestone, magnesian limestone, oolite, and dolomite.

Chalk waters are mostly bright, transparent, and charged with CO_2 . When the CO_2 is driven off, these waters are almost universally alkaline, although before boiling the reaction to litmus may be distinctly acid. Chlorine varies from 2 to 3 parts, nitric nitrogen from 0.2 to 0.4, total hardness 15 to 30 (the hardness is chiefly temporary, and may be nearly all due to carbonates of Ca), and total solids from 25 to 50 parts.

Waters from oolite closely resemble those from chalk, with the exception that they contain a little more permanent hardness. Limestone waters contain more total solids and more permanent hardness (due principally to calcium and magnesium sulphate). Waters from dolomite strata occupy an intermediate position between chalk and limestone waters in point of hardness and total solids. Greensands, in that they frequently contain much nitrates and variable quantities of ferrous iron, furnish, through the reduction of the nitrates by the iron, quantities of free NH_3 . The intermediate stage of nitrites may be occasionally demonstrated. The lower greensand furnishes water collected at great depth—often many feet below the chalk—and accordingly the total solids are high, often 80 to 100 parts per 100,000. Hardness is very variable, and much is permanent. Chlorine may run to 10 or 12 parts per 100,000, and nitric N to as much as 0.5 or 0.6. These waters are very free from

organic matter. Where water is procured from lias clays much permanent hardness may be expected (CaSO_4 and MgSO_4), 20 parts or more, and total solids may range from 200 to 300.

A water containing over 30 parts of total hardness may be considered unsuitable for domestic purposes, unless it can be largely softened.

Waters containing more than 20 parts of permanent hardness are not suitable for washing and cooking.

Deep wells, if sufficiently steined, are for the most part pure. Very occasionally a well in the chalk may tap a hidden reservoir of unpurified sewage which has leaked through fissures from a cess-pool.

Sewage derives the bulk of its Cl from urine, which contains, as above mentioned, about 1 per cent. chlorides, but although it contains this large amount of Cl, it is obvious that deadly pollution by sewage may occur in such small amounts as are wholly incapable of detection by chemical methods; the chlorine figure, therefore, will be chiefly of diagnostic value in those cases where the soil, subsoil, and water-bearing strata are of constant composition and beyond the reach of contamination by cultivated land. If after a series of analyses the Cl figure is found fairly constant, a particular rise of 0.5 to 1 part per 100,000 may justly arouse a suspicion of sewage pollution.

Considering the varieties in source and surrounding conditions from source to distribution, it is quite impossible to erect standards of purity for waters in this country. An inspection of the source and surroundings is of the utmost importance in all cases.

In considering the 'free and saline' NH_3 , the merest trace should be considered of import if not suspicious, except in those cases where reduction of nitrates has taken place, such as occurs in the green-sands. As previously stated, if the 'albuminoid' ammonia be very small (less than 0.002), the 'free and saline' may be allowed to exceed slightly 0.005. In peaty waters, where the 'albuminoid' ammonia may reach 0.01, the 'free and saline' should be negligible. In a deep-well water the O absorbed from permanganate in 3 hours at 37° C. should not exceed 0.01 or 0.02. In a peaty water free from animal pollution this figure may exceed 0.1.

In passing judgment on river waters, analyses, in addition to

inspection, should be made of all tributaries, lest evidence of present or past pollution be overlooked. The search for poisonous metals should be carefully carried out, and when any of these is found a quantitative estimation should invariably be made. Lead to the extent of 0.025 part per 100,000 is sufficient to condemn a potable water. Present or recent sewage pollution may be more or less accurately differentiated from past and remote, in that, whilst high Cl and nitrate figures obtain in both, in the present or recent contamination there will be marked free and organic NH_3 , whereas in the past and remote little or no free or organic NH_3 will be found. Further animal pollution may be more or less accurately differentiated from vegetable by contrasting the two ammonias, oxygen absorbed by Tidy's process, Cl, and nitrates. All these figures are high in cases of marked animal pollution; whilst in vegetable pollution free NH_3 is low, organic NH_3 high, Cl and nitrates are low, and in the last two no increase if the water is drawn from below the surface. Where much vegetable matter exists the water is usually coloured, as in the various peaty waters, and the solid residue chars on ignition. Sulphates and phosphates occur in larger quantities in water polluted with animal matter than in those contaminated with vegetable material. Little has been said of nitrites, because, although they are easily formed by oxidizing and reducing agents, they are rarely present in natural waters. They are found in purifying sewage, but, unfortunately, as they may be formed from other sources than ammonia (such, *e.g.*, as nitrates in contact with iron, zinc, and lead pipes or cisterns), it is not always possible to locate their origin. The faintest trace, however, of nitrites should condemn a water, except in the single instance of a pure water containing nitrites undergoing reduction by metallic or other inorganic compounds, and not by organic matter.

The solids impart different properties to waters according to their composition, so that no strict limit can be set to their amount. Sulphates should not exist in larger quantity than 8 parts SO_2 per 100,000. Magnesium salts, especially MgSO_4 , should be very small, if at all present, in a good water. And perhaps, all forms of mineral matter considered, the total figure should not nearly reach 100.

CHAPTER VII

THE BACTERIOLOGY OF WATER

THE student who works with a microscope should be familiar with the elementary mathematics of the instrument; he should understand the principles which underlie the formation, magnification, and brightness of images. The following matters require special attention: (1) The conditions which produce an aplanatic image as expressed in Abbé's sine law—in other words, the conditions which exclude spherical aberration and coma. (2) The angular and numerical aperture of a lens and their relations to the refractive indices of glass, air, cedar-wood oil, etc. (3) The meaning of resolution as applied to lenses and the factors determining its limits. (4) The definition of lenses. (5) Methods of excluding chromatic aberration. (6) The flatness of images. (7) The theory of the Huygenian eyepiece. (8) Methods of illumination including oblique or dark ground illumination. (9) The simple relations between objectives (high and low power), Abbé condenser, mirror (plane and concave), diaphragm, and source of light.

The student approaching the bacteriology of water is assumed to have a good bench knowledge of—

1. The preparation and examination of the hanging drop with a view to determination of motility, immotility, and Brownian movement.

2. The preparation and staining by a simple stain of a smear or section with the object of discovering the morphology of the micro-organism or micro-organisms under examination—coccus, bacillus, vibrio.

3. The preparation of a Gram specimen. He ought to be able to definitely state whether his specimen is positive or negative.

4. The few special stains—*e.g.*, Ziehl Neelsen's, used for *Bacillus*

tuberculosis and acid-fast bacteria; Neisser's, used for the Klebs-Löffler bacillus, etc.

5. The preparation of and results obtained by the various fermentation media in common use, especially those for intestinal bacteria.

6. The methods employed in carrying out immunity reactions between micro-organisms and blood serum.

The bacteriological examination of water as a routine procedure seeks (1) to measure the extent to which it has been polluted by sewage; or (2) to determine the degree of completeness of purification processes; or (3) to detect the presence of definite disease-producing organisms, such as *B. typhosus*, etc. Since the number of definite pathogenic organisms compared with the total number of bacteria in water is very small, and since competition may have wholly eliminated the disease-producers by the time the water reaches the laboratory, the search under head (3) becomes so unsatisfactory that it is but rarely attempted. The search under head (2) is most serviceable in determining the efficiency of sedimentation and filtration of large quantities of water. The micro-organisms characteristic of sewage generally styled 'indicator' organisms—viz., *B. coli*, streptococci, and *B. enteritidis sporogenes*—when estimated quantitatively determine with considerable accuracy the degree of sewage pollution remaining at any stage in the purification of a water-supply, and to the expert in charge this piece of bacteriological evidence is of the first moment. But the search under head (1) is that most widely engaged in.

B. Coli.

Of the three indicator organisms above named, *B. coli* is by far the most important; so universally is this recognised that the bulk of bacteriological examinations of water is limited to a quantitative determination of this organism alone. We have in the *B. coli* group bacteria extremely numerous in excreta and sewage, but which do not occur in air, soil, or water unless these have been in contact with sewage.

It is difficult to define the characters of the group. All its members are non-sporing short bacilli, Gram negative, motile, although motility is not always seen, fermenters of glucose and

lactose with production of acid and gas, and fail to liquify gelatin in fourteen days. Attempts have been made in recent years to differentiate the strains of *B. coli* found in human excreta from those of the domestic and other animals. At present it is impossible to distinguish *B. coli* isolated from water as belonging to any species of animal. Whether or not *B. coli* of intestinal origin can be definitely separated from *B. coli* of soil, etc., is a matter of much difference of opinion. The broad landmarks that separate the fermentation reactions of *B. coli* from those of *B. typhosus* and *B. enteritidis* (Gärtner) necessarily disappear when varieties of *B. coli* are to be distinguished. Under favourable conditions *B. coli* may persist for considerable periods outside the intestinal tract which is its natural habitat; but under ordinary conditions it disappears rapidly from soil, water, etc. This last statement is vindicated by the self-purification of rivers from *B. coli* carried into them by sewage, and by experimentally applying sewage to soil, water, etc., and determining the dates at which *B. coli* can no longer be found. Whether it be safe to rely on fine distinctions in fermentative reactions and on pathogenic and agglutination properties as means for separating *B. coli* of recent intestinal origin—the type most clearly indicative of danger—from organisms that have persisted in water, soil, etc., after typhoid bacilli or cholera vibrios have perished, is a question which all water investigators have to face, and until it can be definitely answered—and that time is not yet—it would appear to be safer to regard all forms of *B. coli* as possible indicators of sewage. Houston some years ago worked out a set of tests represented by the symbol 'flaginac' to assist in distinguishing *B. coli* of intestinal origin—viz.:

| | |
|--|-------|
| Greenish fluorescence in neutral red broth | = fl. |
| Acid and gas in lactose peptone media | = ag. |
| Indol in broth or peptone water | = in. |
| Acidity and clotting in litmus milk | = ac. |

Later he modified his procedure somewhat and adopted the following three tests for *B. coli*, using in each case portions of water measuring 100 c.c., 10 c.c., 1 c.c., 0.1 c.c., 0.01 c.c., and 0.001 c.c.:

1. *Presumptive*.—Gaseous fermentation of a bile salt glucose peptone medium.

2. *Confirmatory*.—Isolation of a coli-like microbe forming gas either in a lactose or glucose medium.

3. *Typical*.—Isolation of a coli-like organism forming indol in peptone water and gas in a lactose medium.

Houston was the first to use the above and lesser dilutions with the object of placing Public Health bacteriology on a combined qualitative and quantitative basis. He used the words 'flaginac' and 'typical' only as an indication that specified tests have been carried out, and did not claim that 'flaginac' or 'typical' *B. coli* are only found in human excremental matter.

Technique of the Search for 'Flaginac' B. Coli.—Remove bottle of water for examination from its case and gently shake it. Remove cork and flame mouth. Sow 100 c.c. of the water into 50 c.c. MacConkey's fluid, triple strength, in a Durham's tube. Sow 10 c.c. into 10 c.c. MacConkey double strength. Sow 1 c.c. into 10 c.c. MacConkey ordinary strength. After forty-eight hours incubation at 37° C. note presence or absence of gas. If gas is found, dilute a loopful of the culture in 10 c.c. sterile water and spread two loopful of the dilution on a surface culture of MacConkey's tauro-chloate-lactose agar for isolation. Examine after forty-eight hours for coli-like colonies. If such be found sow one or two in a tube of liquefied glucose-gelatin and incubate at 20° C. If gas be formed liquefy the gelatin and use it for sowing neutral red broth, peptone water, and litmus milk. Examine for fluorescence, indol, and acid, and clot respectively. An organism giving all these reactions is said to be 'flaginac' or 'typical' *B. coli*.

Streptococcus Group.

There does not appear to be any uniform classification of streptococci. Morphology, pigment production, agglutination tests, pathogenicity, and production of acid in sugars have all been recommended as bases for classification. But for water examination attempts to differentiate isolated streptococci have been up to the present wholly unsuccessful.

Technique of Search for Streptococci.—Sow 1 c.c. of the water into 10 c.c. of ordinary broth. Incubate at 37° C. After forty-eight hours examine the deposit microscopically for streptococci.

B. *Enteritidis Sporogenes* (Klein).

This bacillus possesses distinctive characters. It is fairly large—2 to 4 μ long by 0.8 μ broad; it is motile; it spores near the ends of the rods; it is Gram positive; it grows anaerobically in milk, producing a characteristic coagulum of casein and a transparent or turbid and acid whey, whilst gas is formed in quantity. The contents of the incubated milk tube smell of butyric acid. When a c.c. of the whey containing numbers of bacilli is injected into the groin of a guinea-pig the animal dies within twenty-four hours, and post-mortem examination reveals an extensive gangrenous slough at the seat of inoculation. These post-mortem appearances, together with the changes in the milk, identify the organism.

As *B. enteritidis sporogenes* is a sporing organism with prolonged powers of resistance it can hardly be regarded as indicative of recent excretal pollution. Indeed, opinion is far from united concerning its value as an indicator of sewage pollution.

Technique of Search for B. Enteritidis Sporogenes.—Sow 10 c.c. of the water into 50 c.c. of milk, taking care to pass the pipette well below the cream. Sow 1 c.c. into 10 c.c. milk. Heat the tubes to 80° C. for fifteen minutes, and then incubate in an anaerobic apparatus at 37° C. The typical 'enteritidis' change consists in formation of gas, odour of butyric acid, separation of curd, and tearing of same by gas.

It is impossible to set up rigid bacterial standards for waters. But the source being known general indications can readily be offered as to what should be expected of a good water. Since the more recent the excremental pollution the greater the number and the older the pollution the less the number of *B. coli* present, the bacteriological potentialities of a sewage-contaminated water would appear to be best expressed in terms of the number of *B. coli* found.

For deep wells and springs a more restricted standard will be demanded than for shallow wells, rivers, upland surface waters, etc.

For deep wells and springs it may be required that *B. coli* and streptococci be absent from 100 c.c., and that *B. enteritidis sporogenes* be absent from a litre; that the growth on gelatin at 22 degrees does not exceed fifty organisms per c.c., whilst that on agar at 37 degrees does not exceed five per c.c.

In shallow wells, rivers, upland surface waters, etc., this standard may be relaxed to one-tenth—viz., absence of *B. coli* and streptococci from 10 c.c., and of *B. enteritidis sporogenes* from 100 c.c.; gelatin growth not to exceed 500 per c.c., and agar not more than 50 per c.c.

Sea water is regarded as polluted by most observers when it contains *B. coli* in 1 c.c. Houston states that no sample of sea water remote from pollution contains *B. coli* or spores of *Enteritidis sporogenes* in 100 c.c. Whilst no absolute standards can be fixed, it may, perhaps, be stated in a general way that samples in which *B. coli* is present in 10 c.c., but absent in 1 c.c., are to be regarded as suspicious.

Technique—Collection of Sample.—1. A white glass bottle, capacity 200-500 c.c., is sterilized and plugged with sterile cotton-wool. 2. Before filling flame the mouth and remove the plug; fill quickly, and insert a new cork which has just been passed through a flame till slightly carbonized. Cut off cork level with mouth and seal with wax. Cover with a rubber cap. In taking water from a river, submerge bottle some distance from bank with mouth upstream; from a tap, let run to waste before filling; from well, lower under same conditions as bucket is lowered, or fill from bucket, or use a Miquel flask.

As organisms rapidly multiply in water at ordinary temperatures the sample should be kept at 0°C. until examination is commenced. Special boxes containing ice are prepared for this purpose.

The label should specify (1) Reasons for examination (epidemic, etc.); (2) source of water; (3) particulars concerning recent rains, snow, pollution, etc.; purposes for which water is required (drinking, cooking, lavatories, etc.); (4) atmospheric temperature; (5) day and hour of collection.

Enumeration of Organisms.—Prepare a few 10 c.c. pipettes plugged at the upper end with wool, and sterilize them; also a drop pipette (20 drops = 1 c.c.).

Sterilize some conical flasks plugged with wool. Liquefy a few tubes of gelatin in a water-bath, and prepare some sterile distilled water.

Measure 9 c.c. sterile water into a flask, taking the necessary precautions to avoid all contamination; to this add 1 c.c. of the

water under investigation, and mix. The mixture is a dilution of 1 in 10.

Flame the mouth of a conical flask; remove the plug; introduce with the drop pipette 2 drops of the 1 in 10 dilution. Flame the mouth of a gelatin tube, remove the plug, and quickly pour the contents into the conical flask; mix, and stand the flask on a cold horizontal surface—ice in hot weather.

A gelatin plate has been made containing 0.01 c.c. of the water.

Incubate this at 20° to 22° C.

Examine the flask daily for appearance of colonies, and make counts until the gelatin is completely liquefied.

Suppose by the fifth day there are 90 colonies, and on the sixth the plate is completely liquefied, record is made that "the water contains 9,000 (100 × 90) aerobic micro-organisms per c.c., liquefaction of the gelatin having finished the count on the sixth day."

Enumeration may be carried out with pipettes (made in France) which deliver about 50 drops to the c.c. The exact number of drops per c.c. is marked on the stem.

In the same manner inoculate melted agar at 40° C. and pour plates; incubate at 37° C. for three days; count.

Qualitative Examination.—Sow 1 drop of the water in a tube of melted gelatin or agar; mix; sow 2 loopfuls of the mixture into a second tube of gelatin or agar; mix; sow 2 loopfuls of the last mixture into a third gelatin or agar tube; pour plates in Petri dishes with these mixtures. The plates are carefully observed daily, and subcultures sown in other media for the identification of a particular colony. Many saprophytes in water, although incapable of causing infections, may, like *Proteus vulgaris* and *Micrococcus prodigiosus*, produce soluble toxins which injuriously affect man and the lower animals; others may cause a nuisance by producing in dead organic matter foul-smelling gases.

The detection of pathogenic species, such as *Bacillus typhosus*, is generally a matter of some labour. When the student has gained facility in technique, he should conscientiously work out the various sugar reactions, growths on special media, and the tinctorial and morphological characters of this and a few other pathogenic forms, such as *B. pyocyaneus*, Friedländer's bacillus, and the micro-

organisms of suppuration. Detailed descriptions of methods must be sought in systematic works on pathological bacteriology.

The various items of the analysis are recorded in some such form as this:

Sample of water from _____ Date _____

Labelled _____

Brief particulars of source _____

Physical characters :

Turbidity _____

Colour _____

Odour _____

Reaction _____

Free and saline NH_3 _____ parts per 100,000.

Albuminoid NH_3 _____

Cl. _____

Nitrous N _____

Nitric N _____

Hardness (total) _____

„ (permanent) _____

„ (temporary) _____

O absorbed at 37°C in three hours _____

Metals _____

Solids (total) _____

„ (volatile) _____

„ (fixed) _____

Appearance on ignition _____

Microscopic examination of sediment _____

Bacteriological Examination _____

EXAMPLES OF WATERS FROM VARIOUS SOURCES

RESULTS EXPRESSED AS PARTS PER 100,000.

| | No. 1.
A Pure Water. | No. 2.
Rain Water Collected
on Grass Land. |
|--|---|--|
| Physical characters - - - | Excellent | Good |
| Reaction - - - - - | Faint alkaline | Faint alkaline |
| Free and saline NH_3 - - - | 0.001 | 0.012 |
| Organic NH_3 - - - - - | 0.001 | Nil |
| Cl - - - - - | 1.200 | 0.200 |
| Nitrous N - - - - - | Nil | Nil |
| Nitric N - - - - - | 0.010 | 0.010 |
| Hardness (total) - - - | 8.500 | 0.600 |
| " (permanent) - - - | 3.000 | 0.600 |
| " (temporary) - - - | 5.500 | Nil |
| O absorbed at 37° C. in three hours | 0.013 | 0.002 |
| Metals (Zn, Pb, Fe, Cu) - - | Nil | Nil |
| Solids (total) - - - - - | 11.500 | 2.500 |
| " (volatile) - - - - - | 2.500 | 1.000 |
| " (fixed) - - - - - | 9.000 | 1.500 |
| Appearance on ignition - - | Nil | Nil |
| Microscopic examination of sedi-
ment - - - - - | Nil | Nil |
| Bacteriological examination - | 20 non - liquefying
saprophytes per
c.c. Intestinal
organisms absent | Not performed |

| | No. 3.
Foul. | No. 4.
Chalk Water from
Deep Well. |
|--|--|--|
| Physical characters - - - | Excellent | Excellent |
| Reaction - - - - - | Alkaline | Alkaline |
| Free and saline NH_3 - - - | 0.030 | 0.005 |
| Organic NH_3 - - - - - | 0.020 | 0.006 |
| Cl - - - - - | 5.000 | 4.500 |
| Nitrous N - - - - - | 0.050 | Nil |
| Nitric N - - - - - | 0.600 | 0.300 |
| Hardness (total) - - - | 20.000 | 22.000 |
| " (permanent) - - - | 12.000 | 12.000 |
| " (temporary) - - - | 8.000 | 10.000 |
| O absorbed at 37° in three hours | 0.150 | 0.060 |
| Metals (Zn, Pb, Fe, Cu) - - | Nil | Nil |
| Solids (total) - - - - - | 30.500 | 38.000 |
| " (volatile) - - - - - | 10.500 | 12.000 |
| " (fixed) - - - - - | 20.000 | 26.000 |
| Appearance on ignition - - | Marked charring | charring |
| Microscopic examination of sedi-
ment - - - - - | Objects indicating
sewage pollution | Nil |
| Bacteriological examination - | <i>B. coli</i> found in
50 c.c. | Not performed |

Sample No. 2, although it possesses a high 'free' NH_3 figure, is good. Rain water in towns is generally impure; it is slightly acid from SO_2 , and contains NH_3 .

Sample No. 3 has had a small amount of untreated sewage admitted to it.

Sample No. 4 is an average chalk water with low total solids. This figure may be allowed to go up to 200 or over. The hardness of chalk waters varies considerably.

Sample No. 5.—The saline NH_3 , chlorine, and nitrates are high, and nitrites are present. These items in general point to animal

| | No. 5.
Deep-well Water from
the Lower Greensand. | No. 6.
Deep-well Water from
Chalk near the Sea. |
|---|--|---|
| Physical characters - - - | Good | Saline taste, greenish colour, no odour |
| Reaction - - - - - | Alkaline | Alkaline |
| Free and saline NH_3 - - - | 0.035 | Nil |
| Organic NH_3 - - - - - | 0.001 | 0.003 |
| Cl - - - - - | 12.250 | 115.000 |
| Nitrous N - - - - - | 0.020 | Nil |
| Nitric N - - - - - | 0.320 | 1.000 |
| Hardness (total) - - - | 16.000 | 47.000 |
| " (permanent) - - - | 10.000 | — |
| " (temporary) - - - | 6.000 | — |
| O absorbed at 37° C. in three hours | 0.020 | 0.035 |
| Metals (Zn, Pb, Fe, Cu) - - - | Nil | Nil |
| Solids (total) - - - - - | 105.000 | 260.500 |
| " (volatile) - - - - - | 20.000 | 35.500 |
| " (fixed) - - - - - | 85.000 | 225.000 |
| Appearance on ignition - - - | Nil | Slight darkening |
| Microscopic examination of sediment - - - - - | Nil | Mineral particles |
| Bacteriological examination - | Excellent | Excellent |

pollution; that they are not due to this cause here is shown by the low organic NH_3 and O absorbed. Reduction of nitrates by iron salts is going on, as demonstrated by the high saline NH_3 and presence of nitrites.

Sample No. 6 is contaminated by sea water. Before contamination Cl was 3, and total hardness 20. Much MgCl_2 is present, and the water is unfit for domestic use.

Sample No. 7 contains much acid, and could not be allowed to traverse lead pipes. Its organic NH_3 and O absorbed are not so high as in many peaty waters.

| | No. 7.
Surface Water, Peaty. | No. 8.
Surface Water, not Peaty. |
|--|----------------------------------|-------------------------------------|
| Physical characters - - - | Colour brownish;
slight taste | Almost colourless,
no taste |
| Reaction - - - | Acid | Neutral |
| Free and saline NH_3 - - - | 0.001 | 0.002 |
| Organic NH_3 - - - | 0.030 | 0.003 |
| Cl - - - | 0.600 | 0.800 |
| Nitrous N - - - | Nil | Nil |
| Nitric N - - - | 0.010 | 0.030 |
| Hardness (total) - - - | 3.000 | 3.000 |
| " (permanent) - - - | 3.000 | 2.500 |
| " (temporary) - - - | Nil | 0.500 |
| O absorbed at 37° C. in three hours - - - | 0.150 | 0.050 |
| Metals (Zn, Pb, Fe, Cu) - - - | Nil | Nil |
| Solids (total) - - - | 9.000 | 4.000 |
| " (volatile) - - - | 2.000 | 1.000 |
| " (fixed) - - - | 7.000 | 3.000 |
| Appearance on ignition - - - | Charring | Faint darkening |
| Microscopic examination of sedi-
ment - - - | Vegetable débris | Nil |
| Bacteriological examination - - - | No intestinal or-
ganisms | No intestinal or-
ganisms |

Samples Nos. 9 and 10 were taken from the same house. The analysis of 10, carried out a month after that of 9, shows some slight variations, which are to be expected, when it is remembered that the composition of river water varies, with its varying powers of

| | No. 9.
New River Water from
the Lea. | No. 10.
New River Water from
the Lea. |
|--|--|---|
| Physical characters - - - | Excellent | Excellent |
| Reaction - - - | Slightly alkaline | Slightly alkaline |
| Free and saline NH_3 - - - | Nil | 0.001 |
| Organic NH_3 - - - | 0.002 | 0.003 |
| Cl - - - | 1.900 | 1.8600 |
| Nitrous N - - - | Nil | Nil |
| Nitric N - - - | 0.160 | 0.210 |
| Hardness (total) - - - | 20.500 | 21.500 |
| " (permanent) - - - | 11.500 | 13.500 |
| " (temporary) - - - | 9.000 | 8.000 |
| O absorbed at 37° C. in three hours - - - | 0.017 | 0.023 |
| Metals (Zn, Fe, Pb, Cu) - - - | Nil | Nil |
| Solids (total) - - - | 32.600 | 28.560 |
| " (volatile) - - - | 10.200 | 10.000 |
| " (fixed) - - - | 22.400 | 18.560 |
| Appearance on ignition - - - | Nil | Nil |
| Microscopic examination of sedi-
ment - - - | Nil | Nil |
| Bacteriological examination - - - | — | — |

self-purification, with the nature of the strata in which its springs of origin occur, and of the strata over which it flows, and with the

| | No. 11.
Peaty Water. | No. 12.
Well Water. |
|--|-------------------------|------------------------|
| Physical characters - - - | Colour light-brown | Excellent |
| Reaction - - - - - | Acid | Neutral |
| Free and saline NH_3 - - - | 0.005 | Nil |
| Organic NH_3 - - - - - | 0.026 | 0.001 |
| Cl - - - - - | 1.500 | 3.800 |
| Nitrous N - - - - - | Nil | Nil |
| Nitric N - - - - - | 0.220 | 0.362 |
| Hardness (total) - - - | 3.500 | 26.000 |
| " (permanent) - - - | 3.500 | 12.300 |
| " (temporary) - - - | Nil | 14.000 |
| O absorbed at 37°C. in three hours | 0.146 | 0.008 |
| Metals (Zn, Fe, Pb, Cu) - - | Nil | Nil |
| Solids (total) - - - - - | 12.300 | 38.260 |
| " (volatile) - - - - - | 8.300 | 8.500 |
| " (fixed) - - - - - | 4.000 | 29.760 |
| Appearance on ignition - - | Charring | Nil |
| Microscopic examination of sedi-
ment - - - - - | Vegetable débris | Nil |
| Bacteriological examination - | Negative | Nil |

nature of the soils and subsoils of its basin, especially in regard to cultivation, density of population, and the presence of sewage and industrial waste.

| | No. 13.
Lambeth Supply. | No. 14.
Chelsea Supply. |
|--|----------------------------|----------------------------|
| Physical characters - - - | Excellent | Excellent |
| Reaction - - - - - | Faint alkaline | Neutral |
| Free and saline NH_3 - - - | Nil | Nil |
| Organic NH_3 - - - - - | 0.005 | 0.002 |
| Cl - - - - - | 1.850 | 1.740 |
| Nitrous N - - - - - | Nil | Nil |
| Nitric N - - - - - | 0.086 | 0.009 |
| Hardness (total) - - - | 18.600 | 18.400 |
| " (permanent) - - - | 8.500 | 8.400 |
| " (temporary) - - - | 10.100 | 10.000 |
| O absorbed at 37°C. in three hours | 0.043 | 0.023 |
| Metals (Zn, Fe, Pb, Cu) - - | Nil | Nil |
| Solids (total) - - - - - | 26.400 | 26.720 |
| " (volatile) - - - - - | 6.800 | 6.400 |
| " (fixed) - - - - - | 19.600 | 20.320 |
| Appearance on ignition - - | Nil | Nil |
| Microscopic examination of sedi-
ment - - - - - | Nil | Nil |
| Bacteriological examination - | Nil | Nil |

Sample No. 11 is plumbo-solvent, and is slightly polluted with animal matter, in that free and saline NH_3 , Cl, and nitric N, are too high for a peaty water.

Sample No. 12 is a pure water from a deep well in Kent.

Samples Nos. 13 and 14 are fair specimens of filtered Thames water.

In the 'nil' returns of the bacteriological and sediment examinations, it is to be understood that nothing was found indicative of animal pollution.

A DORSET WATER-SUPPLY.

Three samples selected from a series of thirteen investigated at the same time by the writer, and showing the changes effected by treatment with chalk and filtration. These waters, derived from Bagshot sands, covered with peat, are acid and ferruginous.

| | (A)
Before Treatment
with Chalk. | (B)
After Treatment
with Chalk. | (C)
After Filtration. |
|--|--|---------------------------------------|--------------------------|
| Physical characters— | | | |
| Colour, smell, turbidity - - | Good | Good | Excellent |
| Chemical reaction - - | Acid | Acid | Acid |
| | Acidity = }
2.190 HCl } | 0.500 | 0.350 |
| Free and saline NH_3 - - - | 0.018 | 0.022 | 0.001 |
| Organic NH_3 - - - | 0.016 | 0.010 | 0.001 |
| O absorbed in three hours at
37° C. - - - | 0.040 | 0.020 | Nil |
| Total solids - - - | 12.300 | 11.500 | 11.500 |
| Hardness (total) - - - | 2.500 | 2.500 | 2.700 |
| " (temporary) - - - | — | — | — |
| " (permanent) - - - | 2.500 | 2.500 | 2.700 |
| Chlorine - - - | 2.500 | 2.000 | 2.500 |
| Nitric N - - - | 0.100 | 0.100 | 0.010 |
| Iron in solution - - - | 0.325 | 0.220 | 0.110 |

As the filtered water still contains acidity, it may not be passed through lead pipes.

Sea water contains in 100,000 parts nearly 2,000 parts Cl, and between 3,000 and 4,000 parts total solids. Hardness ranges between 500 and 600 parts. Lime and magnesia together form about 240 parts, and the ratio of the first to the second is about 1 : 6.

The following is an estimation:

| | | | | | | | In 100,000 pts |
|-------------------------------|---|---|---|---|---|---|----------------|
| Free and saline NH_3 | - | - | - | - | - | - | 0.006 |
| Total solids | - | - | - | - | - | - | 3,380.000 |
| Lime | - | - | - | - | - | - | 35.000 |
| Magnesia | - | - | - | - | - | - | 205.000 |
| Silicia | - | - | - | - | - | - | 0.450 |
| Hardness | - | - | - | - | - | - | 580.000 |
| Chlorine | - | - | - | - | - | - | 1,875.000 |

The table below represents the comparative figures for the principal chemical constituents of a well-filtered river water, delivered to a town of some 40,000 inhabitants, and the sewage of the same town before treatment:

| | Water. | Sewage. |
|---|--------|---------|
| Free and saline NH_3 - - - | Nil | 6.800 |
| Organic NH_3 - - - | 0.002 | 2.000 |
| O absorbed in two hours at 80° F. - - - | 0.020 | 4.080 |
| Chlorine - - - | 1.850 | 11.800 |
| Nitric N - - - | 0.120 | Nil |
| Total solids - - - | 28.500 | 160.000 |

CHAPTER VIII

SEWAGE EFFLUENTS

AN average sample of the day's working should in all cases be obtained, and the analysis performed forthwith.

It has been usual to estimate the 'free and saline' and 'albuminoid' NH_3 , O absorbed from permanganate, total solids, solids in solution, suspended matter, oxidized N, and Cl.

The physical characters may be noted, and incubation at 37°C . for forty-eight hours may be effected in order to determine the presence or absence of further fermentation, as indicated by odour.

The analysis is frequently required for the determination of the degree of purification at a particular stage, or the comparative value of a certain method of sewage treatment. It is usual to record the purification as percentages of the figure for albuminoid NH_3 . If, for example, before treatment the albuminoid figure is 0.6 part per 100,000, and after treatment 0.15, it is clear that purification has taken place to the amount of 0.45 part per 100,000, or 75 per cent. of the original albuminoid NH_3 has been oxidized.

The ammonias are estimated as described under water, but a large dilution of the effluent is necessary; 10 c.c. may be made up to 1,000 c.c. with distilled water, and in some instances 5 c.c. in the same volume will be convenient.

The O absorbed from permanganate is estimated by Tidy's process, and care should be taken that sufficient permanganate is added from time to time, and that the flask is frequently shaken. A convenient dilution is 10 c.c. in a litre.

In the working out of this process it should be noted that various bodies besides organic matter absorb O from permanganate, such as nitrites, sulphites, sulphides, sulpho-cyanates, numerous dyes, and various coal-tar products.

Total solids are estimated by evaporating 100 c.c. of the sample in a platinum dish on a water-bath. When dry, the dish is transferred to an air-bath, and dehydration continued. It is then passed through a desiccator, and weighed. The difference between this weight and that of the dish represents the 'total solids.'

The solids in suspension are found by passing 100 c.c. of the sample through two folds of filter-paper, whereby the solids in solution alone pass through. The filtrate is evaporated to dryness, further dehydrated, desiccated, and weighed. The result represents the 'solids in solution.' The difference between this weight and that of the total solids represents the 'solids in suspension.'

In estimating nitrous N, dilute the liquid with distilled water (free from nitrite) to a convenient strength. Take 100 c.c. in a Nessler glass, as described under water, add 1 c.c. metaphenylenediamine and 1 c.c. H_2SO_4 (1 in 3). Match by treating in a similar manner a standard solution of potassium nitrite made up to 100 c.c. Stand for twenty minutes before comparing.

The nitric N is estimated by Crum's method or by the copper-zinc couple. A convenient dilution must be made. Where time is an item, as in examinations, the less accurate phenol sulphonic acid method may be used.

If raw sewage is to be analyzed, weaker dilutions must be used—5 c.c. or less in a litre.

In the distillations carried out in Wanklyn's process the volume of the boiling fluid should never be allowed to fall below 150 c.c. Hot, ammonia-free distilled water when necessary should be added.

Griess's test should be promptly performed, and if the fluid is not transparent it should be filtered before adding the reagents.

Estimation of the total N by Kjeldahl's method is a much more accurate index of the organic pollution than that by Wanklyn's process for albuminoid ammonia. The latter usually gives less than half the N figure obtained by the former.

With a little practice Kjeldahl's method can be carried out rapidly and accurately as follows:

In a Kjeldahl flask put 10 c.c. sewage effluent and 1 c.c. H_2SO_4 , and evaporate on a water-bath to half the bulk. When cool, add about 10 c.c. oil of vitriol, and about 10 grammes sulphate or bisulphate of potassium (to raise the boiling-point). Digest under a hood in a draught-chamber. Continue the digestion until the solu-

tion is a pale, transparent yellow colour—*i.e.*, until all the C has been completely oxidized. Cool and wash out into a distilling-flask. Make up to 500 c.c. with ammonia-free distilled water; add excess KOH and a piece of ignited pumice-stone, and distil over nearly 350 c.c. Nesslerise the ammonia collected, and subtract from the result the amount of free and saline ammonia previously estimated. The difference is the NH_3 due to organic nitrogen. The reagents used should be free from NH_3 .

Digestion with concentrated H_2SO_4 converts the N into $(\text{NH}_4)_2\text{SO}_4$. Subsequent addition of excess of KOH decomposes $(\text{NH}_4)_2\text{SO}_4$, with liberation of NH_3 , which is distilled over.

Instead of Nesslerising, the NH_3 can be received in excess of standard acid, and the unsaturated acid finally titrated with alkali: the amount of acid saturated is equivalent to the NH_3 from which the N is at once calculated.

The purification of sewage has little influence on the amount of its chlorine, which in average samples reaches 10 or 11 parts per 100,000.

A sewage effluent should be colourless and without odour.

The albuminoid NH_3 should not exceed 0.1 to 0.15 part per 100,000, nor the O absorbed in four hours at 37°C . 1 to 1.5 parts. Cl and free and saline NH_3 are unimportant.

The pouring of crude sewage or badly purified effluents into rivers of limited volume will cause deoxidation of the water, with consequent injury to fish and other forms of aquatic life, putrefaction of organic matter with resulting nuisance, growth of sewage fungus, disposition of suspended matters, etc.

In order to determine the condition of contaminated streams in respect of odour, development of grey algæ, accumulation of putrefying sewage solids, and injury to fish life, the Sewage Commissioners (1898, still sitting) have confined their attention mainly to three tests:

1. The amount of ammoniacal N.
2. The amount of O absorbed from permanganate in four hours.
3. The amount of dissolved O taken up in five days.

Whilst the ammoniacal N may be considered as the most delicate chemical index of recent sewage pollution, it is not equally reliable in demonstrating the character of the pollution as indicated by the effect which the sewage produces on the stream. The nuisance-producing power of a sewage or effluent is broadly proportional to its power of deoxygenating the water of the stream, and tests based on

the rate and degree of absorption of O are the most trustworthy for determining whether or not nuisance is likely to occur in a stream. The five days' test represents naturally the actual process by which the more readily oxidizable constituents of the polluting matter absorb the O dissolved in the river-water, and shows smaller differences in quality of water.

The permanganate process may give, approximately, the same figure for a water polluted with tank liquor and for a water polluted with filter effluent, while the five days' dissolved O test will give a higher figure for the water polluted with tank liquor, thus indicating differences in kind as well as in degree of pollution.

The Commissioners conclude that if 100,000 c.c. of river water do not take up more than 0.4 gramme dissolved O in five days, the river will be free from signs of pollution; but that if it takes up a higher figure it will most probably show signs of pollution. This number 0.4 they term the limiting figure, and regard it as the best foundation on which to construct a scheme of standards.

As results will be found to vary according to temperature, they adopt the temperature of 65° F. (18.3° C.), and in order to provide a wide margin of safety the dry weather flow of the river.

It will be seen that the amount of dissolved O taken up in five days by a mixture of river water and sewage depends—(1) on the amount taken up by the sewage liquor; (2) on the amount taken up by the river water; (3) on the proportion in which the two liquids are mixed.

If x = parts of dissolved O taken up per 100,000 by sewage;
 y = parts of dissolved O taken up per 100,000 by river water
 above outfall;
 z = dilution (proportion of river water to sewage);

$$\text{then } \frac{x + yz}{z + 1} = 0.4.$$

Thus, if an effluent which takes up 0.1 part dissolved O in five days be discharged into ten times its volume of water, we get—

$$\frac{x + (0.1 \times 10)}{10 + 1} = 0.4$$

$$x + 1 = 4.4$$

$$x = 3.4$$

—that is, in this case, the effluent may be allowed to take up 3·4 parts dissolved O per 100,000 in five days, which figure would be the standard for this particular discharge.

The most important local condition is the degree of dilution afforded by a river to the contaminating discharge. It is advised that a standard effluent should not contain more than 3 parts suspended solids per 100,000, and that samples which satisfy this test must also be considered in relation to the five days' test. The latter is fixed at 2 parts per 100,000.

An effluent which takes up 2 parts per 100,000 dissolved O in five days will need some dilution if nuisance is to be avoided. The minimum degree of dilution required for safety can be found from the formula:

$$\frac{2 + (0.2 \times z)}{z + 1} = 0.4,$$

$$z = 8.$$

It is considered safe to assume that the majority of effluents are diluted by more than eight times their volume of river water.

It is recommended, therefore, that an effluent should not contain more than 3 parts suspended matter per 100,000, and that, including its suspended matter, it should not take up more than 2 parts dissolved O per 100,000 in five days at 18·3° C. It is suggested that this be considered the normal standard for effluents. An effluent is considered satisfactory that contains less than 3 parts per 100,000 suspended solids, and which, after filtration, does not absorb in parts per 100,000 more than 0.5 dissolved O in twenty-four hours, or 1.0 part in forty-eight hours, or 1.5 parts in five days.

Adeney's method of determining the rate of absorption of dissolved O by polluted waters is described in detail in the Fifth Report of the Royal Commission on Sewage Disposal.

The Report (Cd. 4,278, 1908) states that effluents which are derived from strong original liquids may often contain large amounts of organic matter in solution, and yet not take up dissolved oxygen rapidly from water or cause injury to the streams into which they are discharged. Such effluents, judged by the empirical tests hitherto in common use, might be regarded as polluting liquids. The effect of an effluent on a stream does not depend on the absolute amount of organic matter in it, but on the nature and condition of

that organic matter, and the important thing to ascertain is the extent to which the original organic matter has undergone fermentation. Dunbar has shown that after a certain percentage purification the residual organic matter in certain sewages is so altered as to be non-putrescible.

To determine the rate of absorption of dissolved O, it is only necessary to ascertain by a volumetric process the amount of dissolved O in the effluent when fresh, and in a portion of the same effluent after it has been kept for a definite period of time—two to five days. The difference between the two estimations will give the amount of O absorbed during the time of keeping, and the rate of absorption may be taken to be uniform, at least for the first two days of observation.

If a knowledge of the attendant changes which take place during the various stages of the fermentation be required, it will be necessary, in addition to estimation of the dissolved O, to determine the NH_3 and HNO_2 and HNO_3 before, during, and after period of fermentation.

For most practical purposes it is only necessary to determine the rate and total absorption of oxygen and the character of the fermentation, whether a carbon or nitrogen one. The first can be done by estimating the loss of O in the atmosphere of the flask containing the polluted water, and the second by ascertaining whether nitrites and nitrates have been formed or not. In most cases, however, as Adeney shows, even this will be found unnecessary, as the completion of the carbon-oxidation stage of fermentation will be indicated by the cessation of the absorption of oxygen which occurs during the interval of rest which takes place before the commencement of the nitrogen-oxidation stage.

The Process.—A measured quantity of the polluted water (100-250 c.c., according to amount of polluting matters contained) is decanted into B, into which a little freshly precipitated magnesium hydrate has been previously placed for the purpose of fixing the CO_2 in the water. A similar volume of distilled water is poured into A. Similar volumes of air are thus left in the two bottles. These volumes should be sufficiently large (capacity, a litre or more) to ensure much more O in B than can possibly be used.

Corks, connecting-tube, and stopcocks are fitted. A slight rise of

capillary water will occur in the portion of the connecting-tube in A. The height of this capillary column is marked with a diamond or file; the mark serves as an index for subsequent measurement. With both stopcocks open the two bottles are immersed in a water-bath for a few minutes to allow of their contents assuming a common temperature. Both stopcocks are then closed, and the temperature of the bath and the height of the barometer are noted.

The bottles are taken out of the water-bath and dried. When completely dry the corks are coated with shellac varnish to prevent

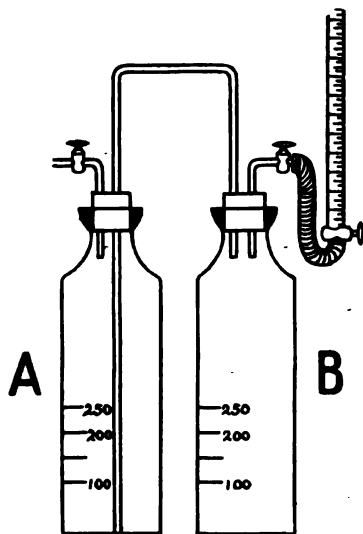


FIG. 23

diffusion of air through them. The apparatus is then put in a mechanical shaker, which keeps the contents in gentle motion.

As O is absorbed by the polluted water in B, the pressure of the atmosphere is reduced relatively to that of the atmosphere in A, which is unaffected by the distilled water. Accordingly, water from A will rise in the connecting-tube in proportion to the volume of O absorbed by the polluted water from the atmosphere in B.

This volume of O can be measured at any time by attaching by means of a flexible tube a burette containing distilled water at the temperature of the laboratory. As the water from the burette is

cautiously allowed to flow into B the water in the connecting-tube will gradually sink back to the index, at which instant the stopcock to B is closed. The reading on the burette is equal to the volume of O, which has been absorbed from the atmosphere of B at the temperature and pressure obtaining at the commencement of the experiment.

The distilled water bottle A acts as a reference pressure bottle. If a comparatively rapid absorption of O occurs during the first hour or two and this is followed by a slower and regular absorption, it may safely be taken to be due to the polluted water being de-aerated to start with, and possibly also to the presence of easily and directly oxidizable substances in it; the subsequent slower and regular absorption being due to indirect oxidation accompanying the fermentation of the polluting matters.

It is very important, as noted above, that sufficient excess of air be always secured, otherwise the operation is open to certain obvious inaccuracies. The replacement of the absorbed O by water is equivalent to increasing the pressure of the N in B, which will lead to absorption of N by the polluted water and the distilled water added to it, unless the air in the bottle be in such large excess that the O absorbed be only a small fraction of it. Further, with insufficient air there may be such a reduction in the store of O in the atmosphere of B as to lead to appreciable reduction in the rate of fermentation in the polluted water.

Bacteriological Examination of Sewage and Sewage Effluents.—Houston's method of water examination is equally suitable for sewage effluents and sewage when these have been properly diluted. In the estimation of *B. coli* it may be necessary to work on as small a quantity as 0.000001 or even 0.00000001 c.c. of sewage.

CHAPTER IX

SOIL

Analysis of Soils.

THE analysis of soils is a large subject, and requires for its proper execution a special training in chemistry. For public health purposes, however, very few estimations are required, and these are of a simple kind. The powers which a soil possesses for absorbing and retaining moisture are of some importance, but direct examination of the soil and subsoil in position in a given locality will furnish more valuable information than laboratory tests.

The capacity for absorbing moisture may be estimated by means of a percolator and burette. A quantity of dried soil (say 100 grammes) is flooded with water for two hours and allowed to drain for four hours. The difference in the reading of the burette before and after the operation gives the number of c.c. of water absorbed by 100 grammes, or the absorption per cent.

Perhaps a simpler method is the following:

One hundred grammes of dried soil are covered with water in a cylinder. Sufficient time is allowed for saturation, which in the case of clay soils may be several hours. The water is drained off through a muslin filter, and the soil is reweighed. The increase in weight roughly represents the percentage absorption.

The determination of the size of the particles of a soil is carried out by using a series of sieves possessing meshes of 2 millimetres, 1 millimetre, and 0.5 millimetre respectively. A number of meshes larger than 2 millimetres may be used.

One hundred grammes of dried soil are pulverized with the fingers. The larger pebbles, roots, etc., are removed and weighed. The residue is transferred to the 2-millimetre sieve, and when all has passed that will, the remainder is further rubbed between the fingers, and

once more shaken on the sieve. What remains on this sieve is weighed. In like manner the amounts left on the other sieves are weighed. Finally, the soil which passes the 5-millimetre sieve is weighed, and the results are collected as (a) coarse masses removed by hand, (b) masses kept back by the 2-millimetre sieve, (c) sand retained by the 1-millimetre sieve, (d) fine sand retained by the 0.5-millimetre sieve, and (e) fine soil passing the 0.5-millimetre sieve.

The specific heat of soils is determined by a sensitive calorimeter. The specific heat ranges from 0.2 to 0.5, and is greatest in peaty soils.

The determination of the porosity of a soil is effected by finding the real and apparent specific gravity of the soil, and dividing the latter by the former.

The real specific gravity is obtained by placing in a 50 c.c. specific gravity bottle 10 grammes of the soil dried at 100° C. to constant weight, rinsing the last particles into the bottle with distilled water, and making up with distilled water to the mark. The whole is weighed at 15° C. The weight of the water displaced by the 10 grammes of soil is thus easily calculated. The weight of the soil—viz., 10 grammes—divided by the weight of displaced water is the specific gravity.

The apparent specific gravity is obtained by filling a 1,000 c.c. cylinder with soil, introduced in small quantities at a time, and thoroughly settled in the cylinder by tapping from time to time on the bench. When full the cylinder is covered with a glass plate and weighed. The weight of the soil (cylinder full – cylinder empty) divided by 1,000 is the apparent specific gravity.

The real specific gravity of a sample was found to be 2.46, and the apparent 1.36; the porosity is therefore $\frac{1.36}{2.46} = 0.55$, or expressed as a percentage = 55 per cent.

Pore volume, or porosity, being the sum total of the interstitial spaces which may be filled with water or air, or both, does not depend on the size of the particles but on their uniformity, or want of uniformity, of size, and on their arrangement. The porosity of a soil composed of uniform spherical particles the size of peas is the same as that of another composed of particles the size of small shot, and in each case is about one-third of the whole.

Permeability of soil to air depends not on the amount of its pore volume, but on the size of the individual spaces. Permeability diminishes to an extraordinary degree with diminution of the size of the particles.

The water retained in soil exists as hygroscopic water adherent by surface attraction to the soil grains, and as capillary water held up in the capillary spaces. The latter constitutes by far the larger portion of the retained moisture. If the texture of a soil be so fine that all spaces are within the limits of capillary magnitude, the maximum water-retaining power is attained.

The differentiation of the constituents of soils into sand, clay, and organic matter is of some importance.

Sand consists of the coarser particles which rapidly sink in water; clay of the fine particles which remain for a time in suspension.

The estimation of sand and clay may be performed thus: Take 10 grammes of dried soil in a beaker. Moisten the soil with a little distilled water and a few drops of a solution of NH_4Cl . When moist add 80 to 100 c.c. distilled water, and stir. Allow to settle for five minutes, and pour off the fluid into a tall cylinder. Another 100 c.c. of water is added, and the soil well stirred. After settling again for five minutes the fluid is poured off into the cylinder. These manipulations are repeated until the overlying fluid is quite clear.

The sand is turned on to a filter-paper, well washed, dried, weighed, and recorded as sand.

The cylinder is set aside for twenty-four hours, and when fully settled the upper portion of the fluid is run through a filter-paper without disturbing the sediment. When nearly all the water has been drained off, the sediment is stirred and poured on the filter. The last traces of clay are washed from the cylinder, and the entire contents now on the filter are thoroughly washed, dried, weighed, and recorded as clay.

Clay soil contains over 30 per cent. clay; some brick clays contain 95 per cent. Sandy soils contain as little as 1 or 2 per cent. clay; a loam contains 10 per cent.

In order to determine the amount of organic matter in a soil, take 10 grammes of a dried sample in a platinum dish, and heat it at a temperature a little over 100°C . until a constant weight is obtained.

Oxidize over a flame at a low red heat, transfer to desiccator, and weigh. The loss in weight gives roughly the amount of organic matter.

Lime may be estimated thus: Dissolve a few grammes of the dried soil in dilute HCl, and dilute the resulting solution to about 100 c.c. with water. Heat, add NH_4OH in slight excess, and a solution of ammonium oxalate also in slight excess. Allow the precipitate to settle in a warm place. Pass the clear liquid through a small filter and then bring the precipitate upon it. Wash with hot water and set the filtrate and washings aside. Push the precipitate and filter-paper through the funnel into a flask, add some H_2SO_4 , dilute freely, warm to 60° or 70° , and run in $\frac{\text{N}}{10}$ permanganate until faint pink remains. Each c.c. of $\frac{\text{N}}{10}$ permanganate represents 0.0028 gramme CaO.

Magnesia.—Evaporate the filtrate and washings to small bulk on the water-bath, render alkaline with NH_4OH , add sodium phosphate, and set aside for eight or ten hours in order that the magnesia may separate out as $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$. Wash this precipitate on to a filter with ammonia solution. Dry in hot-air chamber and ignite to form $\text{Mg}_2\text{P}_2\text{O}_7$, from which the weight of MgO is easily calculated.

Or the ammonio-magnesium phosphate precipitate may be brought upon a filter washed with ammoniacal water in the cold, dissolved in acetic acid, and titrated with standard uranium solution, each c.c. of which represents 0.002815 gramme magnesia.

The phosphoric acid in soils is determined as follows: (1) Incinerate and digest a weighed quantity of the soil with HCl, evaporate to dryness to render silica insoluble, redigest with acid, filter, and wash. (2) Concentrate the filtrate and washings to small bulk and add excess of ammonium molybdate in nitric acid, stand aside in a warm place for two days, decant the liquid through a filter, wash the precipitate several times by decantation first with dilute HNO_3 , and afterwards with small amounts of distilled water, then transfer it to the filter and wash free from excess of acid, dissolve the ammonium-phospho-molybdate in ammonia, add magnesium mixture, filter, wash, dry, and ignite the precipitate. Weigh the resulting $\text{Mg}_2\text{P}_2\text{O}_7$, from which calculate the P_2O_5 .

Or the method described for magnesia may be used, wherein the ammonio-magnesium phosphate precipitate is dissolved in acetic

acid, and titrated with standard uranium acetate or nitrate solution, each c.c. of which equals 0.005 gramme P_2O_5 .

The total organic nitrogen of soil is best estimated by Kjeldahl's method. The ammonia resulting from the distillation of the ammonium sulphate with excess of KOH is received in $\frac{N}{10}$ or $\frac{N}{20}$ H_2SO_4 . At the end of the distillation the standard H_2SO_4 remaining is titrated with standard alkali, and the ammonia absorbed by the acid calculated. The N forms $\frac{1}{7}$ by weight of the NH_3 .

Where total N (including HNO_2 and HNO_3) is required the oxidized N must first be converted into NH_3 by boiling with Al and NaOH, or by the action of the Cu-Zn couple.

Clay and humus are the two most important ingredients of soils. The plasticity and adhesiveness of clay, together with the fineness of the particles, serve to hold together various other aggregates of soil. The extreme fineness of the particles of clay causes it to retain water, solids dissolved in water, and gases.

If by plastic or colloidal clay be understood the particles of soil under 0.01 millimetre diameter which remain suspended in a column of water eight inches high for twenty-four hours, soils may be divided into the following six classes:

| | | | | | |
|---|---|---|-------|---|-------------|
| Very sandy soils containing up to 3 per cent. clay. | | | | | |
| Sandy | " | " | 3-10 | " | " |
| Sandy loams | " | " | 10-15 | " | " |
| Clay | " | " | 15-25 | " | " |
| Clay soils | " | " | 25-35 | " | " |
| Heavy clays | " | " | 35-45 | " | " and over. |

Admixture of fine powders, such as $Ca(OH)_2$ and $Fe_2(OH)_6$, diminish greatly the adhesiveness of clay, caused by the hydrated silicates.

'Humus' or 'vegetable mould' is formed by the decomposition of organic matter, largely cellulose, derived from the roots, stems, and leaves of plants. Its accumulation near the surface is natural, and it distinguishes soil from subsoil. Its production is controlled by moisture, oxygen, temperature, and micro-organisms. With a low temperature and as much water as will shut out air the organisms that transform vegetable tissue into humus are bacteria; but the disinfectant compounds produced soon kill the bacteria, and the process remains henceforth a slow and purely chemical one. In

the solid brown decomposition products formed in peat are found ulmic and apocrenic acids soluble in caustic and carbonated alkalies, and forming insoluble salts with the earths and metals, and ulmin, insoluble in alkalies but afterwards soluble on oxidation. CO_2 and CH_4 are formed in large quantities under these conditions. Prolonged cultivation of soils tends to production of acids; hence the advantages of calcareous formations. In the presence of earthy carbonate, especially that of lime, which neutralizes acids as formed, moderate degrees of moisture, and free circulation of air, humification proceeds under the influence of moulds instead of bacteria. O and H are eliminated as CO_2 and H_2O , and an increase takes place in the percentage of C and N. When humification is complete and oxidation proceeds, the N may rise to high figures, portions being wholly oxidized to nitrates.

Humus is highly porous, absorbs water and gases, and is gradually oxidized by bacteria. The measure of this oxidation can be gauged by the amount of CO_2 produced. Humus substances are gelatinous when moist, but not markedly adhesive or plastic. The density of humus is about 1.4; hence soils rich in humus are light (humus is the lightest ingredient of soil) when compared with clay and sandy soils, and 'light' in the agricultural sense of being easily tilled.

The N of humus does not exist in the form of NH_3 , as it cannot be set free by treatment in the cold with lime or alkalies. When humus is boiled with lime or alkalies ammonia is slowly evolved for an indefinite time, but the whole of the N is not expelled. Such behaviour, together with its slightly acid reaction, points to humus being of the nature of an amido-compound.

Humus formed from sugar, cellulose, gums, etc., combines with ammonia as with other bases, and at first the ammonia can be readily expelled from this as from other ammonia salts. But after a time the amidic condition appears to be assumed, as caustic alkalies act but slowly, and are unable to expel the whole of the N. These facts are of importance in nature, as NH_3 , generated in or taken up by the soil, is in the course of time rendered inert and unavailable for plants until nitrification has been effected.

Humin and ulmin found in the deeper layers of peat are in process of time oxidized into humic and ulmic acids capable of combining with bases. Further oxidation produces crenic and apocrenic

acids, readily soluble in water and capable of uniting with bases to form salts. These acids react on decomposable silicates and dissolve them; they also dissolve ferric hydrate. In this way rust-coloured soils are bleached by stagnant water and deprived of much of their mineral plant food.

In ordinary soils humus rarely exceeds 5 per cent., in peat and marsh lands it may reach 20 per cent.

Humus may be estimated by extracting the soil with dilute acid to set free the humic bodies from their combinations with lime and magnesia. The residue is then extracted with moderately dilute solutions of ammonia. Evaporation of the ammonia extracts leave the humus as a black lustrous substance (*matière noire* of Grandeau). As this contains a variable amount of ash it is burnt and the ash is subtracted from the first weight.

A determination of the ash of humus gave:

| | | | | | | | |
|---|---|---|---|---|---|---|-------|
| Insoluble matter (principally silica) - | - | - | - | - | - | - | 62.6 |
| K ₂ O | - | - | - | - | - | - | 7.5 |
| Na ₂ O | - | - | - | - | - | - | 8.1 |
| CaO | - | - | - | - | - | - | 0.1 |
| MgO | - | - | - | - | - | - | 0.3 |
| Fe ₂ O ₃ | - | - | - | - | - | - | 3.1 |
| Al ₂ O ₃ | - | - | - | - | - | - | 3.4 |
| P ₂ O ₅ | - | - | - | - | - | - | 12.3 |
| SO ₃ | - | - | - | - | - | - | 0.9 |
| CO ₂ | - | - | - | - | - | - | 1.7 |
| | | | | | | | 100.0 |

Bacteria of Soil.—Inseparably correlated with humus and carbohydrates in soil are varied forms of bacteria. More than 40,000,000 per c.c. have been found. The bulk of soil bacteria reside near the surface, as there alone are to be found the conditions necessary to growth and multiplication. The best foodstuffs appear to be water, proteins, and soluble carbohydrates derived from decaying plants, stable manure, etc. When the decaying matter reaches the stage of humus, only few bacteria remain. The most important functions of soil bacteria are related to putrefaction, nitrification, denitrification, and nitrogen-fixation.

Most of the putrefactive bacteria are concerned with the breaking down of complex protein matter, with evolution of NH₃, and forma-

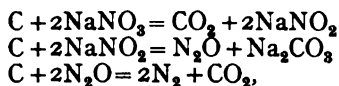
tion of ammonium salts. Common examples of soil organisms of this type are—*Bacillus mycoides*, *B. subtilis*, *B. mesentericus vulgatus*, *Proteus vulgaris*, *P. zenkeri*, *Bacillus coli*, *B. putrificus*, *B. lactis aerogenes*, *B. fluorescens liquefaciens*, streptococci, etc. In acid soils rich in humus certain fungi such as *Penicillium glaucum*, *Mucor mucedo*, and species of *Botrytis* and *Torula* accomplish the cleavage of proteins.

Nitrification is carried out in two stages: ammonium compounds are oxidized to nitrites by such organisms as Winogradsky included in the genus *Nitrosomonas europæa*; and nitrites are oxidized to nitrates by several forms included in the genus *Nitrobacter*. The conditions necessary to these changes are definite. In addition to nitrifiable material and nitrifying bacteria, a fairly high temperature (24° C.), a moderate degree of moisture, free access of oxygen, a base or its carbonate with which the acids formed in the process of oxidation can unite, free CO_2 , and darkness are essential. In acid soils nitrification ceases, as also in soils in which the bases have become fully saturated. Carbonates of lime and magnesia are the bases most favourable to nitrification, and excess of these produces no injury. The amounts of carbonates of potash and soda must be strictly limited. The nitrifying organisms are strictly aerobic; in non-porous or water-logged soils they quickly die out. They derive their carbon from CO_2 , as when cultivated in the presence of carbonates in an atmosphere washed with KOH they fail to develop.

Various denitrifying bacteria have recently been studied. One of the most effective organisms is Burri's *B. denitrificans*, found on the surface of old straw and in fresh horse-dung. If some fresh horse-dung be placed in a close-flask containing KNO_3 , nitrogen and carbon dioxide are evolved, and in a few days the nitrate has disappeared. *B. butyricus*, which in the absence of easily reducible compounds evolves free nitrogen, reduces nitrates to nitrites, and also forms NH_3 by addition of H to N just set free by reduction. *B. mycoides* forms ammonia from antecedent proteins, and also reduces nitrates to nitrites and ammonia. Reduction may be to nitrites, and no further; it may go on to ammonia; nitrates and nitrites may be reduced with evolution of NO and N_2O ; and, finally, nitrates and nitrites may be reduced with production of free N. A

large number of bacteria found in faecal matter, water, and soil decompose nitrates with evolution of free N.

The reactions between nitrates undergoing denitrification and organic carbon compounds may be represented by the equations:



where C represents the oxidizable carbon of the carbon compounds.

An important group of soil bacteria is found in connection with the root nodules of leguminous plants.

The mode of supply of nitrogen to plants was long a subject of debate. Liebig thought that it was derived from the ammonia in rain water. Boussingault proved that plants do not take N directly from the air. Lawes and Gilbert confirmed Boussingault's conclusions. Hellriegel and Wilfarth pointed out that the tubercles on the roots of leguminous plants are produced by bacilli which absorb free N from the air, and pass it over to the host. Beyerinck later separated and described the *B. radiculicola*.

The tissues of one of these nodules on microscopic examination are found to contain a number of free motile bacteria, and a number of quiescent forms much larger in size. When the nodule has reached full size, the large quiescent bacteria begin to collapse, and part with their nitrogenous substance. Later the shells drop off and carry minute bacteria into the soil, which in due course again become active. The nodules adhere but loosely to the roots. The ease with which they may fall off doubtless accounts for the difficulty experienced in transplanting legumes.

The nodules above mentioned vary in shape and size, according to the species of leguminous plant to which they are attached, and are caused by the *Bacillus* or *Pseudomonas radiculicola* (Beyerinck) penetrating the root hairs. On entering root hairs the organism develops and forms a thread-like zooglea technically known as the 'infection thread,' which resembles the hypha of a fungus, and which excites the neighbouring cells of the rootlet to rapid multiplication and the formation of the nodule. In the infection threads and youngest nodules the organisms are straight rods. In older parts they are branched and curved, and are known as bacteroids which

have lost their power of division: later they are digested by a proteolytic enzyme secreted by the protoplasm of the root. The digested substances pass to the flowers and seeds of the ripening plant. N-fixation reaches a maximum at the time when the plants begin to flower. When the crop is harvested, a large surplus of nitrogen is left behind in the nodules in the soil.

Other bacteria are known to absorb free N, of which may be mentioned Winogradski's *Clostridium pastorianum* and Beyerinck's *Azotobacter*.

The nitrogen-fixing powers of soil may be determined by estimating the total N in, say, 200 grammes of soil, and repeating the experiment after six weeks' incubation at 20° C. in a solution composed of grape sugar 40 grammes, K_2HPO_4 2 grammes, NaCl 2 grammes, $CaCO_3$ 10 grammes, and water 2 litres.

The nitrifying and denitrifying powers of soils can be estimated in the same manner by adding known quantities of ammonium salts, nitrites, and nitrates, respectively, to a suitable inorganic medium containing a soluble carbohydrate.

When the surface soil is wetted, moisture may rise toward the surface from the lower layers; this is probably due to evaporation from below, followed by recondensation by the cool wetted layer. The condition is of some practical interest, inasmuch as cold rain on the surface may raise water from below.

The downward percolation of water is most rapid in those soils in which capillary ascent is quickest—*i.e.*, in coarse sand.

The rapidity of percolation decreases as the wetted soil column increases in depth; as the wetted column lengthens, the frictional resistance increasingly opposes the effects of the hydrostatic pressure from above until downward movement becomes little more than lateral movement or capillary ascent from below. The frictional resistance has counteracted gravity to such a degree that the capillary coefficients of the soil become the governing factors of the water movement.

It is often desirable to protect a soil from excessive evaporation in order either to prevent lowering of temperature or to save vegetation in time of drought. The preparation by tilth of a layer of loose dry surface soil is the best means of securing this object. It would appear on first sight that such a soil admits of ready access of air,

and therefore of evaporation; whilst this is true, it is equally true that the coarse particles are incapable of withdrawing moisture from the denser layers beneath in the same manner as a dry sponge is incapable of withdrawing moisture from a wet brick, notwithstanding the fact that a dry brick will readily absorb all the water from the relatively large pores of a wet sponge.

The disinfectant action of dry soil and its capacity for absorbing offensive gases have long been known. The decolorization by soil of drainage from manure-heaps, dye-works, and tanneries, and the filtration of drinking waters on the large scale by fine sand, are equally familiar. It should not be forgotten, however, that these powers are strictly limited. Dry soils are powerful gas-absorbers, and peat appears to excel all others in this property.

The temperature of the soil is derived from the sun's rays, chemical changes in the soil, and from the heat of the earth's interior. The first of these is the chief factor in influencing temperature. The more perpendicularly the rays strike the soil the greater the amount of heat received.

The colour, composition, moisture, and compactness of the soil influence the temperature. A black surface absorbs heat rays more than a white one. Snow melts more rapidly when covered with soot.

Sands and mineral substances in general conduct heat better than water, air, or organic matter. Organic matter is a poor conductor of heat; hence the more humus a soil contains the more slowly will it respond to the action of the sun. Moisture influences soil temperature through the high specific heat of water and through the disappearance of heat due to evaporation. The specific heat of ordinary dry soils is about one-fifth that of water. The drier a soil, the less the evaporation and the greater its warmth.

Vegetation protects against excessive heating in hot climates, and loss of heat in cold climates. Trees impede wind currents and obstruct the sun's rays, so that less loss of moisture occurs by evaporation. It may be quite calm in the centre of a wood whilst a gale blows outside.

A soil in which the ground-water is high—say 5 to 10 feet from the surface—has long been regarded as unfavourable to health. Such a soil renders the atmosphere damp, and appears to conduce

to rheumatism and diseases of the respiratory tract. Lowering of the ground-water level by drainage has largely improved the health conditions of many soils. Soil dampness appears to be connected with pulmonary tuberculosis.

Typhoid fever, cholera, dysenteries, and other intestinal maladies have been etiologically related by various observers to soil, ground-air, and ground-water: it is probable that each and all of these act as media of conveyance of the specific micro-organisms of these diseases.

Newsholme regards epidemics of diphtheria as intimately related to dry years, and holds that they do not occur when the rainfall is above the average.

Malaria is connected with soil conditions in the breeding of the specific mosquitoes.

Ankylostomiasis or uncinariasis is intimately connected with the soil, in that the eggs of the parasite *Ankylostomum duodenale* escape with the fæces and are deposited in the soil, where they hatch in twenty-four hours. The embryos shed their skin twice, and after a few weeks are ready to infest man. The chief portal of infection is the mouth. Several observers assert that the parasites can reach the intestine through the skin.

The various theories which connected goitre with particular constituents of the soil, such as metallic sulphides, magnesian limestone, etc., are now practically abandoned.

Bacteriological Examination of Soil.—This examination is of service principally in connection with water-supplies, more especially contamination of water by surface washings. Much work has been done on *B. typhosus* in soils, and findings have been very varied. Under favourable conditions it appears that this organism can survive for a considerable time.

The organisms of tetanus and malignant oedema are widely distributed in cultivated soil. They are isolated anaerobically from small quantities of soil or soil washings in the usual way: Advantage is taken of the fact that their spores survive heating at 80° C. for a quarter of an hour, when all non-sporing organisms are destroyed. These spores are grown on various media over alkaline pyrogallic solution, and the growths investigated in the usual manner.

In collecting soil for examination, the depth from which the material is to be recovered having been decided upon, a sterile instrument is used for procuring six to twelve specimens, which are mixed in order to produce an average sample. This is carried to the laboratory in a sterile vessel.

A gramme is shaken up in 100 c.c. sterile water in a sterile flask, and from this dilutions are made—1 c.c. of this solution is transferred to 100 c.c. sterile water in a second flask, etc.

Quantitative and qualitative estimations of *B. coli*, streptococci, and *B. enteritidis sporogenes* are carried out in these liquid preparations in the same manner as in dealing with water.

B. coli is absent from uncontaminated soils, or present in very small numbers only. Houston finds that it is not readily isolated even from polluted soils unless the contamination is recent and large in amount. He considers the spores of *B. enteritidis sporogenes* indicative of contamination, but not necessarily recent. Streptococci are found in minimum quantities of soil recently polluted with sewage. They disappear extremely rapidly.

CHAPTER X

AIR

THE air is a mechanical mixture of gases. One hundred volumes contain, roughly, 21 of oxygen, 78 of nitrogen, and 1 of argon, krypton, helium, neon, zeon, and carbon dioxide.

A distinguishing property of gases is that a mass of gas introduced into a closed vessel always completely fills the vessel, however large. Consider two vessels of equal volume connected by a tube carrying a tap, and let one of these vessels be filled with a gas and the other exhausted; on opening the tap, the gas rushes into the exhausted vessel until the same quantity of gas exists in each vessel. Close the tap, and once more exhaust one of the vessels; on opening the tap, the gas expands and again fills equally the two vessels. The operation may be repeated indefinitely, and the gas will always exert some pressure on the inside of the containing vessel.

The density of a gas, like the density of any other body, is the mass of unit volume, and is sometimes referred to hydrogen and sometimes to air as unity at 0° C., and under a pressure of one standard atmosphere.

The only elasticity of which a gas is capable is that of volume or bulk, since it is alone to a change of volume that a gas offers any permanent resistance.

If the pressure on volume V of a gas be increased from P to $P + p$, and as a consequence the volume be reduced from V to $V - v$, the temperature remaining constant, then the strain produced in volume V is v , and per unit volume $\frac{v}{V}$, and the corresponding stress is p . Therefore, since the elasticity of a body is the ratio of the stress to the strain, the elasticity of the gas is $p \div \frac{v}{V}$, or $p \frac{V}{v}$.

By compressing air with mercury in a U-tube closed at one end,

Robert Boyle found a series of values for the volume of a given mass of air under different pressures, and he enunciated in 1662 a law known by his name—viz., that (the temperature remaining unchanged) $PV = \text{constant}$. Mariotte fourteen years later enunciated the same law.

That all gases have the same coefficient of thermal expansion was first enunciated by Charles. Consider a mass of gas of volume v_0 at pressure p_0 , and imagine its volume kept constant while its temperature is lowered from 0°C. to $-t^\circ$, the pressure p will by Charles's law be given by

$$p = p_0(1 - at),$$

where a is the coefficient of expansion. If the cooling be continued to a temperature $\frac{-1^\circ}{a}$,

$$p = p_0(1 - 1) = 0,$$

i.e., at this temperature the gas would exert no pressure on the walls of the containing vessel. According to the kinetic theory of gases, this can only occur when the velocity of translation of the molecules is zero. This temperature is called the absolute zero.

Taking a as $\frac{1}{273}$ (the mean value for hydrogen between 0° and 100°C.), the absolute zero will be -273°C. In order to convert temperatures referred to 0°C. to the corresponding temperatures referred to the absolute zero, it is only necessary to add 273. If T and t represent respectively the absolute and the ordinary temperature,

$$T = t + 273.$$

By Charles's law—

$$p = p_0(1 + at),$$

and $v = v_0(1 + at),$

substituting for a its numerical value,

$$p = p_0[1 + \frac{1}{273}(T - 273)],$$

$$= \frac{p_0 T}{273},$$

$$\text{and } v = \frac{v_0 T}{273}.$$

At any other temperature T^1 , if when the volume is constant the pressure is p^1 , and when the pressure is constant the volume is v^1

$$p^1 = \frac{p_0 T^1}{273},$$

$$v^1 = \frac{v_0 T^1}{273},$$

$$\therefore \frac{p}{p^1} = \frac{T}{T^1}, \text{ and } \frac{v}{v^1} = \frac{T}{T^1};$$

or the pressure at constant volume varies directly as the absolute temperature, and the volume at constant pressure varies directly as the absolute temperature.

A **Barometer** is an instrument used for measuring the pressure exerted by the atmosphere. Barometers may be divided into two classes: (1) Those in which the pressure is measured in terms of the height of a column of a liquid; (2) aneroid barometers, in which the pressure is measured by the strain produced in the lid of a metal box.

Mercury is practically always used in liquid barometers on account of its great density rendering the height of the column supported by the atmosphere a convenient quantity with which to work. Further, mercury does not, as does glycerin, absorb moisture from the air; it has a fairly low freezing-point, and a high boiling-point.

The simplest form of barometer is the siphon barometer, consisting of a U-tube, the longer limb (86 centimetres) of which is closed while the shorter is open. The tube is filled with mercury; by boiling the mercury any air or moisture adhering to the mercury or bore of the tube is expelled. The distance between the levels of the mercury in the two limbs is the barometric height. When the pressure increases, the mercury falls in the open limb and rises in the closed by the same amount, so that the difference of level is double the rise in the closed end or fall in the open. If a scale be attached to either tube, and each inch or centimetre, as the case may be, be marked half an inch or centimetre, the reading at once gives the height of the barometer.

In the Fortin barometer the scale is graduated in inches to 0.05, and the vernier usually reads to 0.002 inch. The cistern is closed below by a leather bag protected by a metal sheath, into the bottom of which is fitted a screw for the requisite adjustments. Having

taken the temperature by the attached thermometer, the mercury in the cistern is raised or lowered by the screw until the ivory point (fiducial point) or zero of the scale and its reflected image in the mercury are just in contact; the vernier is then moved by the upper milled head until its lower edge just excludes the light from the top of the mercurial column; the reading is then made from the scale and vernier.

Verniers are of different lengths, and contain variable numbers of divisions. A common form is $1\frac{1}{2}$ inches long, divided into twenty-five parts, which correspond in length with twenty-four divisions of the principal scale.

A division on the principal scale is therefore greater than one on the vernier by

$$\begin{aligned} & \left(\frac{1}{24} \times 1\frac{1}{2} \text{ inches}\right) - \left(\frac{1}{25} \times 1\frac{1}{2} \text{ inches}\right) \\ &= \frac{25 - 24}{600} \times 1.2 \text{ inches} \\ &= 0.002 \text{ inch.} \end{aligned}$$

To read the vernier adjust its lower edge with the top of the meniscus, when two very small triangles of light will appear, one on either side. If the lower edge of the vernier correspond with a division of the principal scale, this is the reading; but if not, it is evident that the interval between the surface of the mercury and the division of the principal scale next below is equal to the difference between the lengths of the divisions of the vernier and principal scales (0.002 inch) multiplied by the number of vernier divisions which intervene between the lower edge (zero of vernier) and that division which exactly corresponds with a division on the principal scale.

Suppose in a given example that the lower edge of the vernier cuts the principal scale between 29.15 and 29.2 inches, and when the vernier scale is examined it is found that its thirteenth division

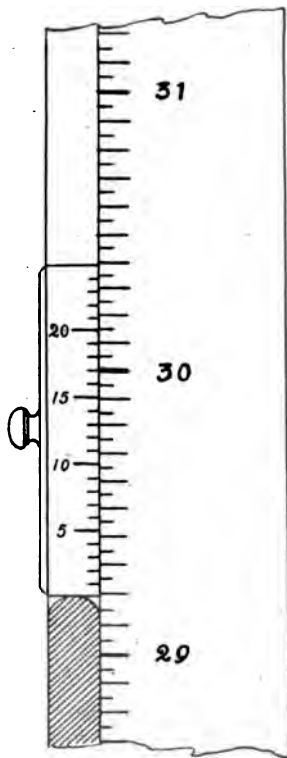


FIG. 24.

corresponds with a division of the principal scale, the reading will be :

$$\begin{aligned} & 29\cdot15 \text{ inches} + 13 \times 0\cdot002 \text{ inches} \\ & = 29\cdot15 \text{ inches} + 0\cdot026 \text{ inch} \\ & = 29\cdot176 \text{ inches.} \end{aligned}$$

The Kew barometer, 'originally invented by Adie for use at sea, has a closed iron cistern, and scale of contracted inches. The tube is of small calibre throughout, in order to lessen the oscillations of the mercury by the ship's motion (known as 'pumping'). A small aperture, covered with leather, in the roof of the cistern, allows atmospheric pressure to exert itself on the contained mercury. Fitzroy's gun barometer is a modification of the Kew.

Hooke's wheel barometer is a siphon barometer. On the surface of the mercury in the lower limb is a float carrying a needle indicator, which moves on a graduated circular dial.

Various self-recording barographs are on the market, records being obtained mechanically, photographically, and electrically.

In order to make an observation of the barometer comparable with other observations taken at other times and places, certain corrections must be applied to it; some of these refer to an individual instrument, and others to all readings of any instrument. Of the former class there are three—corrections for index error, capacity, and capillarity. Of the latter class there are also three—corrections for temperature, altitude, and gravity.

The index error is made by the workman who laid off the scale of the instrument. It is discovered when the instrument is verified at Kew or elsewhere. Correction for capacity depends on the proportion borne by the sectional area of the tube to that of the cistern. At one point of the scale the reading is correct; when the mercury is above that point the correction is additive, when below subtractive.

Capillarity between glass and mercury tends to depress the mercury, and in larger degree the smaller the tube; it is also greater in an 'unboiled' than in a 'boiled' tube. All certificates from Kew for 'Kew' pattern barometers give a correction at each $\frac{1}{4}$ inch, including the above three corrections.

Corrections independent of the Special Instrument.—*Temperature.*—If the scale by means of which the height of the column is measured be correct at 0° C., then at all temperatures above 0° the length of the divisions will be too great, since all metals increase in

length when heated. Let a be the coefficient of linear expansion of the metal of which the scale is made, so that unit length of the scale at 0° C. becomes $1+at$ at t° C. If ht is the reading at temperature t , then the height as measured with the scale at 0° would be greater, since the length of each division of the scale would be less in the ratio of 1 to $1+at$, so that the number of divisions corresponding to a given length (length of mercury column) will be increased in the ratio $1+at$ to 1 .

If h_0 be the barometer reading corrected for expansion of the scale, $h_0 = ht(1+at)$. But h_0 is the height of a column of mercury at temperature t , and the problem is to find what the height would be if the temperature were 0° C. If d_t be the density of mercury at t° , d_0 the density at 0° , δ the coefficient of cubical expansion of mercury, and H the height which the column would have if the mercury stood at 0° C., then 1 c.c. of mercury at 0° becomes $1+\delta$ c.c. at 1° , and $1+\delta t$ c.c. at t° . Since the mass M of the mercury remains unchanged $M = v_0 d_0 = v_t d_t$, where V_0 and V_t = volumes of mass M at temperatures 0° and t° respectively.

$$\therefore d_0 = (1+\delta t)d_t; \text{ or } \frac{d_t}{d_0} = \frac{1}{1+\delta t} = 1 - \delta t + \delta^2 t^2 + \text{etc.}$$

Since δ is excessively small, its second and higher powers may be neglected, and $\frac{d_t}{d_0} = 1 - \delta t$.

Since the height of a column of liquid supported by a given pressure is inversely proportional to the density,

$$\frac{H}{h_0} = \frac{d_t}{d_0} = 1 - \delta t.$$

$$\therefore H = h_0(1 - \delta t) = ht(1 + at)(1 - \delta t) = ht(1 - (\delta - a)t),$$

if δat^2 , which is excessively small, be neglected.

For mercury, $\delta = 0.000182$; for brass, $a = 0.00002$.

Therefore, for a mercury barometer with a brass scale, the corrected height corresponding to an observed height ht at temperature t° C., is given by

$$H = ht(1 - 0.000162t).$$

Altitude and Gravity.—If g = acceleration of gravity at place of observation, and g_{45} that at latitude 45° and at sea-level, l = latitude of observation, and f = height above sea-level,

$$\frac{g}{g_{45}} = 1 - 0.0026 \cos 2l - 0.0000002f.$$

If H_0 be the height under standard conditions corresponding to the same pressure as does H at the place of observation,

$$Hg = H_0 g_{45}; \text{ or } H_0 = \frac{Hg}{g_{45}} = h_1(1 - 0.000162t)(1 - 0.0026 \cos 2l - 0.0000002f).$$

If a bubble of air be passed into the vacuum of a barometer, the mercury falls; if several bubbles be passed in, each produces a depression. If instead of air a drop of ether be introduced, the mercury also falls and the ether becomes completely vaporized, even at a temperature much below its ordinary boiling-point. If successive drops of ether be introduced, it will be found after a time that further addition of ether fails to produce further depression, and that the ether does not vaporize, but floats on the top of the mercury. Now, if the space above the mercury be enlarged or diminished by raising or lowering the barometer-tube in the cistern, it will be found that so long as any liquid ether remains, the height of the mercury column is constant, but that the amount of ether which vaporizes varies with the space above the mercury. If the temperature be increased, more ether vaporizes, and the mercury column becomes more depressed. The vapour exerts a pressure which partly balances the pressure of the atmosphere. The depression of the mercury measures this *vapour pressure*. When excess of liquid is present, so that the vapour exerts its maximum pressure, the vapour is said to be saturated. If, on the other hand, more liquid would vaporize on introduction to the vacuum the vapour is said to be unsaturated or superheated. The vapour pressure, or tension of a liquid, depends on temperature only. Non-saturated vapours obey Boyle's and Charles's laws only approximately, approximation being the more complete the further the vapour is removed from its saturation-point.

Altitudes are calculated from barometric readings either (1) by Laplace's formula, or (2) by Apjohn's formula.

Laplace's formula is—

$$D = 18,363 (\log P - \log p) \left(1 + \frac{2t + t'}{1,000}\right),$$

where D = difference in altitude in metres of the two stations.

P = barometric pressure in mm. Hg at lower station.

p = " " " " " higher "

t = temperature in °C. at lower station.

t' = " " " " " higher "

Apjohn's formula is:

$$D = \frac{16,000 (P - p)}{P + p} \times \left(1 + \frac{2t + t'}{1,000} \right),$$

where D = difference in altitude in metres of the two stations.

P = barometric pressure in mm. Hg at lower station.

p = " " " " higher "

t = temperature in °C. at lower station.

t' = " " " " higher "

Thermometers.—The freezing-point of a thermometer is determined by surrounding the bulb with a mixture of ice and distilled water. The boiling-point is fixed by suspending the instrument in steam issuing from water boiling at a pressure of 760 mm. of Hg. The tube is then calibrated between these two points into 100° in the Centigrade instrument.

Errors of Mercury Thermometers.—The observed expansion is really the difference between the expansion of the mercury and of the glass surrounding the mercury. As different kinds of glass do not expand exactly alike, thermometers made of different glasses do not completely agree. Owing to the gradual recovery of the glass from the effects of the heating to which it was subjected when the thermometer was made, the zero-point rises, at first rapidly, later slowly.

One of the oldest forms of self-registering thermometers provided with a contrivance to mark the highest or lowest temperature obtaining in a given interval of time, is that of Six, made in the eighteenth century. It consists of a glass tube bent twice at right angles, and furnished with a bulb at each end. The bulbs are filled with spirit, except that a bubble of air is placed in the smaller one. The bends of the tube are occupied by a column of mercury. Two steel pins sealed in glass tubes have hairs attached to them, so that they may retain any position reached by being pushed by the mercury column. A magnet is employed to set these indexes. When the temperature rises, the spirit in the large bulb expands, and pushes the index and column of mercury before it. When the temperature falls, the spirit contracts, and the pressure of the air-bubble in the small bulb drives the column of mercury back, which in turn pushes the minimum index before it as soon as the temperature falls below that at which the instrument was set. The defects of the instrument are—it must always be kept in the vertical position, otherwise

the spirit may pass the mercury at the bends of the tube. The mercury tends to pass beyond the ends of the indexes so that small quantities are retained by them.

Modern maximum and minimum thermometers are now always distinct instruments. The student is advised to study these by personal inspection at the show-rooms of a good meteorological instrument maker.

Rutherford's maximum thermometer consists of an ordinary mercury thermometer, with an iron index introduced into the bore (mercury does not wet iron). With rise of temperature the index is pushed before the column of mercury; with fall of temperature the mercury at once parts company with the index. The liquid of the minimum thermometer is alcohol, and the index glass (alcohol wets glass). When the temperature rises, the alcohol flows past the index without moving it; when it falls, the index is carried by the retreating surface of the alcohol by capillarity.

In estimating the weight of volumes of air and aqueous vapour at varying temperatures and pressures, it is necessary to understand aright the meaning of 'density,' 'specific gravity,' and 'relative density.' Density is defined as the mass of unit volume (mass being the amount of matter as measured by inertia); specific gravity is the ratio of the weight of a certain volume at a given temperature and pressure to the weight of an equal volume of a standard substance at the same temperature and pressure. Since the unit volume is 1 c.c., and the unit mass 1 gramme, it follows that water is the standard substance whose density is unity. When the density of oxygen is spoken of as 16, it is meant that the specific gravity of oxygen is 16, hydrogen being taken as the standard; the real density (mass of 1 c.c. O) is 0.0014 gramme. It is preferable to use the phrase 'relative density' of oxygen, etc., and consider it as meaning the same thing as specific gravity when air or hydrogen is the standard. The atomic weights of gaseous elements such as H, N, etc., represent their relative densities, whilst the relative densities of compound gases are represented by half their molecular weights. The relative density of O is 16, that of CO_2 22, H being the standard. The relative density of air referred to the same standard is 14.47.

In hygiene it is customary in calculating the weights, etc., of gases

to take air as the standard. The relative density of H, is therefore $\frac{1}{14.47}$; of O, $\frac{16}{14.47}$; of CO₂, $\frac{22}{14.47}$; and of water vapour, $\frac{9}{14.47}$.

In the metric system the weight of a litre of H at 0° C. and 760 millimetres Hg = 0.0896 gramme.

In English measure the weight of a cubic foot of air at 32° F. and 30 inches Hg = 566.86 grains.

The pressure of water vapour increases with its temperature until at boiling-point it equals that of the atmosphere (30 inches Hg).

The following figures are extracted from a table of vapour tensions:

| | | | |
|--------|---------|------|-----------|
| 32° F. | = 0.181 | inch | pressure. |
| 33° F. | = 0.188 | " | " |
| 34° F. | = 0.196 | " | " |
| 42° F. | = 0.267 | " | " |
| 43° F. | = 0.277 | " | " |
| 44° F. | = 0.288 | " | " |
| 50° F. | = 0.361 | " | " |
| 52° F. | = 0.388 | " | " |
| 53° F. | = 0.403 | " | " |
| 54° F. | = 0.418 | " | " |
| 62° F. | = 0.556 | " | " |
| 63° F. | = 0.576 | " | " |
| 64° F. | = 0.596 | " | " |

Example.—Find the weight of a cubic foot of aqueous vapour at 62° F.

At this temperature the tension or pressure is 0.556 inch. As the relative density of aqueous vapour (air being the standard) is $\frac{9}{14.47}$, or 0.622, it is necessary to find the weight of a cubic foot of dry air at 62° F. and 0.556 inch pressure, and multiply the result by 0.622.

The weight of a cubic foot of dry air under these conditions of temperature and pressure will be given by finding the fourth term x of the proportion:

$$\begin{array}{l} 521 : 491 :: 566.86 \text{ grains} : x \\ 30 : 0.556 \end{array}$$

$$x = \frac{491}{521} \times \frac{0.556}{30} \times 566.86 = 9.9 \text{ grains,}$$

and this multiplied by $0.622 =$ weight of aqueous vapour $= 6.16$ grains.

The pressure of aqueous vapour is constant for a given temperature, whether it is *in vacuo* or mixed with a gas or gases, and varies directly, as has already been stated, as the temperature.

Two other types of problem arise in connection with this subject—namely, (1) finding the weight of a volume of air saturated with vapour at a given temperature and pressure; and (2) finding the weight of a volume of air partially saturated with vapour at a given temperature and pressure.

Example 1.—Find the weight of a cubic foot of air saturated with aqueous vapour at 62° F. and 30 inches Hg.

By the table of vapour tensions it is seen that 62° F. corresponds with 0.556 inch Hg. As the total pressure of air and vapour is 30 inches, the pressure exerted by the air alone must be $30 - 0.556$, or 29.444 inches. The problem, therefore, resolves itself into finding the weight of a cubic foot of dry air at 62° F. and 29.444 inches, and that of a cubic foot of aqueous vapour at 62° F. and 0.556 inch.

$$\frac{491}{521} \times \frac{29.444}{30} \times 566.86 = 524.32 \text{ grains (weight of dry air).}$$

$$\frac{491}{521} \times \frac{0.556}{30} \times 566.86 \times 0.622 = 6.16 \text{ grains (weight of aqueous vapour).}$$

\therefore the cubic foot of saturated air

$$= 524.32 + 6.16 \text{ grains} = 530.48 \text{ grains.}$$

Example 2.—Find the weight of a cubic foot of air partially saturated with aqueous vapour at 62° F. and 30 inches, dew-point being 50° F.

The dew-point is the temperature of complete saturation of the atmosphere. If the atmosphere be raised in temperature, its capacity for holding aqueous vapour will be increased; if lowered, this capacity will be diminished. When the temperature is lowered below the dew-point, vapour is deposited in the fluid form.

Vapour tensions in the above table correspond with temperatures of complete saturation or dew-points, hence, in problems of the type

under consideration, if the dew-point be not given, it must be found.. This may be done directly by such instruments as Daniell's or Regnault's hygrometers, or indirectly by Glaisher's formula, or by Apjohn's formula.

In the indirect method the wet and dry bulb thermometer are used. By Glaisher's formula the dew-point = $D - G(D - W)$ where D = temperature of dry bulb, G = Glaisher's factor for reading of dry bulb, and W = temperature of wet bulb.

By Apjohn's formula :

For temperatures above 32° F.:

$$P = p - \left(\frac{d}{87} \times \frac{h}{30} \right).$$

For temperatures below 32° F.:

$$P = p - \left(\frac{d}{96} \times \frac{h}{30} \right).$$

Where

P = pressure of aqueous vapour at dew-point.

p = pressure of aqueous vapour at temperature of wet bulb.

d = difference in degrees F. between dry and wet bulbs.

h = height of barometer in inches.

Returning to the problem, when the dew-point 50° F. has been found, the pressure of the aqueous vapour 0.361 inch is obtained from the table of vapour tensions.

The problem is resolved as before into two portions—viz., the weight of dry air at 62° F. and pressure 30 - 0.361 inches, and the weight of vapour at 62° F. and pressure 0.361 inch.

$$\frac{491}{521} \times \frac{29.639}{30} \times 566.86 = 527.8 \text{ grains.}$$

$$\frac{491}{521} \times \frac{0.361}{30} \times 566.86 \times 0.622 = 3.98 \text{ grains.}$$

$$527.8 + 3.98 = 531.78 \text{ grains.}$$

Relative humidity represents the ratio between the weight of aqueous vapour present in a given volume of air, and the weight of vapour which would be required to saturate the same volume of air under similar conditions of temperature and pressure, and is expressed as a percentage.

Consider the last example, in which the temperature is 62° F. and the dew-point 50° F.:

Relative humidity=

$$\frac{\text{pressure at } 50^{\circ} \text{ F.} \times \frac{30}{30} \times \frac{49\text{I}}{52\text{I}} \times 566.86 \times 0.622}{\text{pressure at } 62^{\circ} \text{ F.} \times \frac{30}{30} \times \frac{49\text{I}}{52\text{I}} \times 566.86 \times 0.622} = \frac{\text{pressure at } 50^{\circ} \text{ F.}}{\text{pressure at } 62^{\circ} \text{ F.}}$$

$$= \frac{0.36\text{I}}{0.566}; \text{ or expressed as a percentage, } \frac{0.36\text{I} \times 100}{0.566} = 64.9 \text{ per cent.}$$

The composition of the air expired from the lungs contrasted with ordinary air demonstrates the invariable nature of the N and the limits of variation of O and CO₂.

| | Ordinary Air. | Expired Air. |
|-------------------------|-----------------|----------------|
| O - - - - | 20.96 per cent. | 16.4 per cent. |
| N - - - - | 79.00 " | 79.0 " |
| CO ₂ - - - - | 0.04 " | 4.6 " |

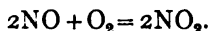
The practically uniform composition of the air all over the earth is maintained by variations of temperature leading to variations of volume and pressure, with resulting air-currents, diffusion of gases, the above-named circulation affected by respiration of animals, transpiration of plants, rain, etc.

Oxygen is the most important constituent of the air, in that it is a prime necessity to life. Its quantity is diminished by respiration, putrefaction, combustions of all types, and at high altitudes.

The estimation of O may be readily carried out in the following ways:

1. The nitric oxide (NO) method.

This method, although it has been adversely criticized, yields, in careful hands, excellent results. The reaction is represented by the equation:



The NO₂ is soluble in water. There is a contraction of three volumes of the mixture for every one volume of O, therefore one-third of the contraction represents the O.

To a sample of air in a gas burette excess of nitric oxide prepared from Cu turnings and HNO₃ is added. The mixture is passed into

an absorption pipette charged with water. The ruddy fumes of NO_2 are rapidly absorbed, and after passing the gas backwards and forwards a few times the reading becomes constant. One-third of the contraction represents the O.

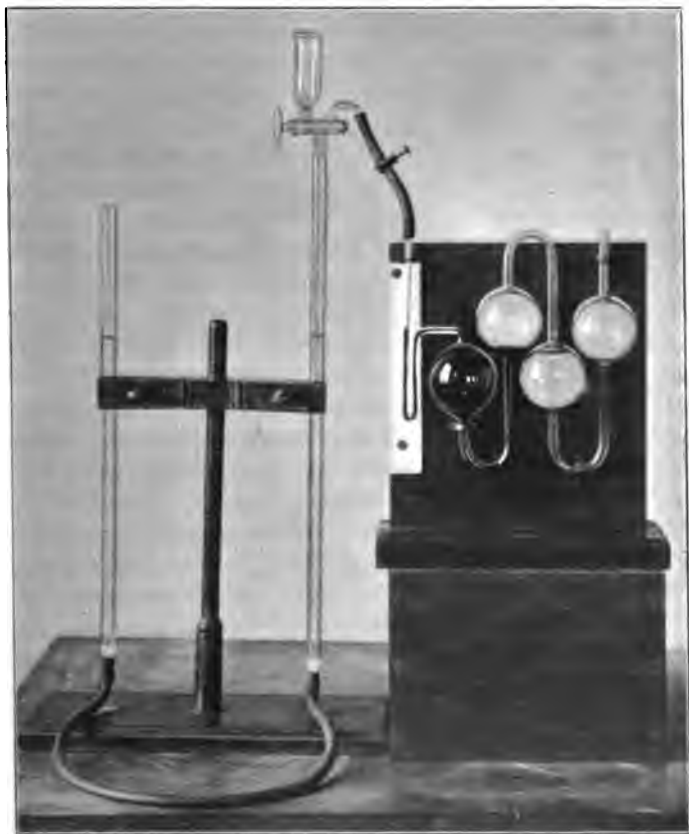


FIG. 25.—HEMPEL'S GAS BURETTE AND ABSORPTION PIPETTE.

2. Hempel's gas burette and absorption pipette.

In the figure the mounted tube—the gas measurer—next to the bulbs is graduated into c.c.'s. and tenths; the other—the levelling-tube—is plain.

The absorption pipette used is a double one, consisting of four

bulbs; the first and largest contains alkali and pyrogallic acid (dissolve 160 grammes KOH in 130 c.c. water, producing about 200 c.c. of solution; in this dissolve 10 grammes pyrogallic acid: if these proportions are not adhered to, evolution of CO may take place during absorption of O, and cause error); the second and fourth are empty; whilst the third contains water to seal off the atmosphere. The reagent absorbs O and CO₂. The graduated burette is supplied at the upper end with a stopcock and a short piece of fine pressure tubing (carrying a screw clip) which connects it with the small manometer U-tube of the bulbs. In order that this piece of tubing may be as short as possible, the bulbs are raised on a block, so that the end of the manometer-tube is near to the burette. The burette and the levelling-tube containing water are connected at their lower ends by rubber tubing.

In making an estimation, first mark on the ivory slip the height at which the coloured liquid stands in the capillary U-tube, turn the stopcock so that connection is made between the burette and bulbs, then raise the levelling-tube until all the air is driven over out of the burette into the bulbs. Now connect the atmosphere with the burette, and lower the levelling-tube until a definite quantity of the particular atmosphere (say 25 or 50 c.c.) is admitted. Then make connection with the bulbs, and raise the levelling-tube until this quantity of air is driven over into the absorption apparatus. Turn the stopcock off, screw down the clip, and unfasten the bulbs from the burette. Shake carefully for ten or fifteen minutes, reunite with burette, and bring back the air by lowering the levelling-tube. Repeat these manipulations until a constant volume is obtained, when the liquid stands at the original mark in the U-tube and the burette is levelled. The decrease in volume is due to the O and CO₂ absorbed. Deduct the CO₂ obtained by Pettenkofer's method, and the remainder represents the O. This volume of O is then reduced to standard temperature and pressure.

Since the temperature should not vary during the operation, the burette must not be handled. The absorption reagent in the first bulb, the water in the third, and the water in the burette, should all be saturated with air before commencing the estimation. It is to be noted that the pyrogallic solution will absorb besides O other gases, such as H₂S, SO₂, HCl, etc.

3. Where accurate estimations are required, the combustion method of Dumas may be used.

A measured volume of air is drawn through KOH to free it from CO_2 , and thence over ignited spongy copper in a combustion-tube. The copper fixes the O, and the amount of the latter is estimated from the difference in weight of the copper and copper oxide.

Carbon Dioxide.—Carbon dioxide may vary in an atmosphere from 0.2 to 0.7 or 0.8 per cent. The quantity ordinarily found in a pure atmosphere ranges from 0.035 to 0.04 per cent.

The atmosphere of London during a fog often contains 0.08 per cent. In a living-room lighted by coal-gas the CO_2 may reach 0.2 per cent., with an appreciable amount of CO.

Carbon dioxide arises from (1) animal respiration; (2) combustion of all kinds of fuel; (3) organic combustion in the form of putrefaction, fermentation, etc. Its special significance lies in the fact that as a product of respiration, it can be made a fairly accurate measure of the organic impurities which accompany it.

Carbon dioxide *per se*, in the quantities commonly found, may be considered harmless. It is generally agreed that the amount furnished by respiration may not exceed 0.02 per cent. Taking 0.04 per cent. as the average quantity found in the air, 0.06 per cent. (0.02 + 0.04) will represent the limit of CO_2 allowable in any atmosphere contaminated by respiration.

The number of cubic feet of fresh air required to dilute the CO_2 of a room, so that this limit may be preserved, will be found by the formula:

$$\frac{\text{cubic feet } \text{CO}_2 \text{ added} \times 100}{0.02}$$

The quantity of CO_2 added to the air through respiration is, roughly, 0.6 cubic foot per head per hour. Substituting this figure in the formula, it is found that 3,000 cubic feet fresh air per head per hour must be admitted to living-rooms if the CO_2 is to be kept within the limits named.

The Estimation of CO_2 in the Atmosphere—Pettenkofer's Method.—When CO_2 is shaken up with baryta water ($\text{Ba}(\text{OH})_2$), insoluble BaCO_3 is formed, and the alkalinity of the fluid is lessened.

Take a 5-litre air-jar, cleansed and filled with water, into the

apartment in which the estimation is to be made. Pour out the water so that the air may enter the jar, and stopper carefully.

Prepare baryta water by adding about 5 grammes Ba(OH)_2 to a litre of distilled water, and accurately estimate, in terms of standard oxalic acid solution, the alkalinity of 25 c.c., using phenolphthalein as indicator. The acid is prepared by dissolving 2.82 grammes of the crystals in a litre. This solution is of such strength that 1 c.c. is equivalent to 0.5 c.c. CO_2 at standard temperature and pressure.

Now add 50 c.c. of the clear barium hydrate solution to the contents of the jar, and roll it round the interior for some time. When, in say twenty minutes, the whole of the CO_2 is absorbed and neutralized, take out 25 c.c. of the solution with a pipette and rapidly titrate it with the standard oxalic acid, delivered from a burette. The difference in alkalinity of this and the original 25 c.c. multiplied by 2 is equivalent to the CO_2 in the jar in c.c. at N.T.P.

Reduce the volume of air in the jar to N.T.P., and calculate the percentage of CO_2 on this.

The following is an example: Temperature 15°C ., pressure 750 millimetres. Twenty-five c.c. of the freshly prepared Ba(OH)_2 were measured by pipette into a porcelain basin, a few drops of phenolphthalein added, and standard oxalic acid run in until the pink colour just disappeared after thorough stirring; 21.5 c.c. of the standard acid were used.

Fifty c.c. Ba(OH)_2 were run into the jar, and after complete absorption of the CO_2 had taken place, 25 c.c. were removed and titrated with acid; 19.9 c.c. standard acid were used. $21.5 - 19.9 = 1.6$ c.c.; and $1.6 \text{ c.c.} \times 2 = 3.2$ c.c. = the total amount of acid equivalent to the CO_2 in the jar. But each c.c. of acid = 0.5 c.c. CO_2 ; therefore $3.2 \times 0.5 = 1.6$ c.c., the volume of CO_2 in the jar, or 1.6 c.c. in 4,950 c.c. (5,000 c.c. - 50 the volume displaced by the Ba(OH)_2). The volume of this 4,950 c.c. at 0°C . and 760° millimetres =

$$\frac{4950 \times 750}{760 \times \{1 + (0.0036 \times 15)\}}$$

(in the C. scale $\frac{1}{273}$ or 0.0036 = coefficient of expansion of gases per degree) = 4,635 c.c.

1.6 c.c. CO_2 in 4,635 c.c. air = 0.03 per cent.

Baryta water is best prepared fresh, but if it must be kept, it should be stored in a vessel shut off from the atmosphere by a hollow tube filled with pumice moistened with KOH. $\text{Ba}(\text{OH})_2$ has a slight action on glass, but any error that might arise through liberated alkalis is so infinitesimal that it may be neglected, especially when a jar has been used a few times.

Angus Smith's 'household test' consists in running $\frac{1}{2}$ ounce of clear lime water into a $10\frac{1}{2}$ ounce bottle. No turbidity will be found so long as the CO_2 in the air does not exceed the limit allowed—viz., 0.06 per cent.

Lunge and Zeckendorf's Method.—A bottle of 70 c.c. capacity and an india-rubber pump of the same capacity are connected so that air can be pumped into the bottle. A weak standard solution of NaOH is prepared and tinted with phenolphthalein, by adding 2 c.c. $\frac{N}{10}$ NaOH containing 1 per mille phenolphthalein, to 100 c.c. ammonia-free distilled water. The ball of the pump is squeezed until the bottle is filled with the air to be tested. Ten c.c. of the $\frac{N}{100}$ NaOH are now placed in the bottle, and the stopper, through which the delivery tube of the pump and an exit tube pass, inserted. The ball is then gently pressed, causing air to bubble through the liquid, and the bottle is carefully shaken after each addition of air until the colour is discharged.

The number of times the ball is emptied indicates the amount of CO_2 , according to the following table, compiled by Lunge from estimations made by Pettenkofer's method:

| | Per Cent. | | Per Cent. |
|------------|-----------|------------|-----------|
| 2 - - - - | 0.3 | 16 - - - - | 0.071 |
| 3 - - - - | 0.25 | 17 - - - - | 0.069 |
| 4 - - - - | 0.21 | 18 - - - - | 0.066 |
| 5 - - - - | 0.18 | 19 - - - - | 0.064 |
| 6 - - - - | 0.155 | 20 - - - - | 0.062 |
| 7 - - - - | 0.135 | 22 - - - - | 0.058 |
| 8 - - - - | 0.115 | 24 - - - - | 0.054 |
| 9 - - - - | 0.10 | 26 - - - - | 0.051 |
| 10 - - - - | 0.09 | 28 - - - - | 0.049 |
| 11 - - - - | 0.087 | 30 - - - - | 0.048 |
| 12 - - - - | 0.083 | 35 - - - - | 0.042 |
| 13 - - - - | 0.08 | 40 - - - - | 0.038 |
| 14 - - - - | 0.077 | 48 - - - - | 0.030 |
| 15 - - - - | 0.074 | | |

When, from respiration, CO_2 rises above 0.06 per cent., a certain unpleasant odour is experienced in rooms, due to the accompanying organic exhalations, and when much above this figure headache and even faintness may supervene. Volatile fatty acids exhaled from the skin and H_2S are responsible for most of these unpleasant odours.

A cubic foot of coal-gas yields on combustion 0.6 cubic feet CO_2 . It is obvious that when CO_2 is due solely to the combustion of coal-gas the quantity may be allowed to exceed considerably the above-named limit.

Carbon Monoxide.—This odourless gas possesses a special affinity for hæmoglobin, displaces oxygen from it, and thus destroys the oxygen-carrying function of the blood and ultimately life, by cutting short internal respiration. When hæmoglobin is saturated to the extent of 30 per cent., symptoms of poisoning set in, and 70 per cent. saturation is fatal. Coal-gas contains by volume about 6 per cent. CO , and when imperfectly burnt leaves small quantities in the flue; but greater danger attaches to the escape of the gas from ill-constructed taps and joints. The use of coke, especially in cast-iron stoves, is a fruitful source of CO . As CO_2 passes over hot coke it is reduced according to the equation $\text{C} + \text{CO}_2 = 2\text{CO}$.

The carbon of the hot cast-iron acts in the same manner, reducing CO_2 to CO . Solid particles of organic matter floating in the atmosphere become charred on the exterior of the stove, and this partial oxidation results in the formation of CO . This gas is present in tobacco smoke.

The characteristic cherry-red colour of CO -hæmoglobin serves as an excellent test for the presence of carbon monoxide. If a few drops of fresh mammalian blood be diluted with water down to about 2 per cent., and the solution shaken up with CO , the distinctive colour is at once formed. If dilution be extended to 0.2 per cent., and HbCO formed by shaking with the gas, the characteristic spectrum consisting of two bands between D and E occupying nearly the same position as those of HbO_2 , but differing in that they do not disappear on the addition of reducing agents such as $(\text{NH}_4)_2\text{S}$ or H_2S , may be readily seen.

HbCO may also be distinguished from HbO_2 by adding to 10 c.c. of the blood solution 10 to 15 c.c. 20 per cent. solution $\text{K}_4\text{Fe}(\text{CN})_6$.

and 2 c.c. acetic acid (1 volume acetic acid + 2 volumes H_2O). A reddish-brown = HbCO ; greyish-brown precipitate = HbO_2 .

The estimation of CO in the air may be performed by (1) Haldane's hæmoglobin percentage saturation method, or (2) by the cuprous chloride method for large quantities.

1. The following is Haldane's account of his method:

'A solution of about 1 of normal blood to 100 of water is made; also a solution of carmine dissolved with the help of a little ammonia, and diluted till its depth of tint is about the same as that of the blood solution. Two test-tubes of equal diameter (about $\frac{1}{2}$ inch) are then selected. Into one of these 5 c.c. of the blood solution are measured with a pipette; into the other about an equal quantity is poured. Ordinary lighting gas is then allowed to blow into the second test-tube through a piece of rubber tubing for a few seconds. The test-tube is then quickly closed with the thumb before the gas has time to escape, and the blood solution thoroughly shaken up with the gas for a few seconds. The hæmoglobin is thus completely saturated with carbonic oxide, and the solution has now the characteristic pink tint. The carmine solution, which has a still pinker tint, is now added from a burette to the 5 c.c. of normal blood solution in the other test-tube until the tints are the same in the two test-tubes. Not only, however, must the tints be equal in quality, but they must also be sensibly equal in depth. If the carmine solution is too strong or too weak, the latter will not be the case, and the solution must be diluted or made stronger accordingly. It is usually easiest to make the carmine a little too strong at first, so that on adding both carmine solution and water equality can be established. From the amount of water which is required to be added it is easy to calculate the extent to which the original carmine solution needs to be diluted. The solutions are now ready for use, and the actual analysis is made as follows: 5 c.c. of the solution of normal blood are measured into one of the test-tubes, and a drop of the suspected blood placed in the other test-tube and cautiously diluted with water till its depth of tint is about equal to that of the normal solution. If carbonic oxide be present in the hæmoglobin, a difference in quality of the tints of the two solutions will now be clearly perceptible. Carmine solution is then added from the burette to the normal blood, and water (if neces-

sary) to the abnormal blood, till the tints are equal in both quality and depth. The carmine is added by about 0.2 c.c. at a time, the points being noted at which there is just too little and just too much carmine, and the mean being taken. The solution of normal blood is then saturated with coal-gas, and the addition of carmine to the other test-tube continued until equality is again established and the amount of carmine noted. The percentage saturation with carbonic oxide of the abnormal blood can now be easily calculated, since we know how much carmine solution its saturation represented as compared with what complete saturation represented.

'The method of calculation is illustrated by the following example: To 5 c.c. of normal blood solution 2.2 c.c. of carmine is required to be added to produce the tint of the blood under examination, and 6.2 c.c. to produce the tint of the same blood fully saturated. In the former case the carmine was in the proportion of 2.2 in 7.2, and in the latter of 6.2 in 11.2. The percentage saturation (x) of the hæmoglobin with carbonic oxide is thus given by the following proportion sum:

$$\frac{6.2}{11.2} : \frac{2.2}{7.2} :: 100 : x.$$

x is therefore = 55.2. As the compound of carbonic oxide and hæmoglobin is, to a slight extent, dissociated when the blood is diluted with water, the value found is a little too low. The corrections needed are as follows: Add 0.5 if 30 per cent. saturation be found, 1.1 if 50 per cent., 1.6 if 60 per cent., 2.6 if 70 per cent., 4.4 if 80 per cent., 10.0 if 90 per cent. Thus, in the above example we must add 1.3, so that the true saturation is 56.5 per cent. In comparing the tints, the test-tubes should be held up against the light from a window, but bright light should be avoided as much as possible, as it increases the dissociation. Failing daylight, an incandescent burner, with a chimney of blue glass and an opal globe, may be used as the source of light.

'Hæmoglobin brought into intimate contact with air containing 0.07 per cent. of CO will finally reach a state of equilibrium in which it is saturated to an equal extent with CO and oxygen. If the percentage of CO or oxygen in the air be increased or diminished, there will be an exactly corresponding increase or diminution of

the relative share of the hæmoglobin which either gas obtains. Air containing $2 \times 0.07 = 0.14$ per cent. of CO will, for instance, produce two-thirds saturation with CO, and one-third saturation with oxygen, and so on. In the living body the proportion of CO taken by the hæmoglobin from respired air containing a given percentage of CO is not so large as outside the body, about 1 per cent. of CO in the air breathed being necessary to produce half saturation of the hæmoglobin. The general law of absorption is, however, much the same, and it follows that there is a certain maximum of saturation for each percentage. With less than 0.05 per cent. of CO in the air this maximum does not exceed 33 per cent. saturation, and the corresponding symptoms are scarcely appreciable, except on muscular exertion. With more than about 0.2 per cent. the maximum exceeds 60 per cent. saturation.

'The detection and determination of small percentages of CO in the air was formerly a matter of great, and often almost insuperable, difficulty. I have recently, however, introduced a simple and, I think, very satisfactory method, depending on the already described action of CO on blood solution in presence of air. The sample of air is collected in a clean and dry bottle of about 4 ounces capacity. The cork of the bottle is removed in the laboratory under a 0.5 per cent. solution of blood, and about 5 c.c. of the air allowed to bubble out, a corresponding volume of the blood solution entering. The cork is then replaced, covered with a cloth to keep off the light, and shaken continuously for about ten minutes, when the hæmoglobin will have reached the point of saturation corresponding to the percentage of CO present. The solution is then poured out into a test-tube, and the saturation is determined with carmine solution in the manner described above. It is evident that as in each case the saturation found corresponds to a definite percentage of CO in the air, it is easy to calculate this percentage. If p be the percentage required, and s the percentage saturation found, p is calculated from the following formula:

$$p = \frac{s \times 0.055}{100 - s}.$$

Thus, if $s = 60$, p is 0.0825. This method may also be used for the direct determination of carbonic oxide in lighting-gas. The latter

must, however, be first diluted to $\frac{1}{100}$ (or with carburetted water-gas to $\frac{1}{100}$) with air. As it is quite easy to make this dilution with perfect accuracy, the method is an exact one, and is not only rapid, but avoids the difficulties and sources of error connected with the ordinary method of determination by cuprous chloride, or by explosion.'

2. The cuprous chloride method.

Cuprous chloride is prepared from copper turnings, copper oxide, and strong HCl, and dissolved in distilled water. This solution absorbs CO.

The air to be treated is first freed from O and CO₂ by passage through Hempel's burette. The residue is slowly and repeatedly passed into a second absorption pipette containing cuprous chloride in solution. The bulb containing the copper salt should be large, the time for absorption long, and the transference from burette to pipette and *vice versa* as often repeated as necessary to procure a constant reading. The loss in volume, assuming that ethylene, acetylene, etc., are absent, represents the CO present. This method is by no means reliable.

Ammonia is found in traces in all atmospheres. It is a product of putrefaction, and although in small quantities it seems to be harmless, it should be regarded with suspicion, by reason of the noxious bodies which accompany it. It is found in larger quantity in air in immediate contact with peat. It may be collected and estimated by aspirating a known volume of air through ammonia-free distilled water, and afterwards distilling and Nesslerising.

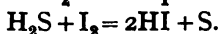
Sulphur Dioxide, Ammonium Sulphide, and Sulphuretted Hydrogen are all present in the atmospheres of cities, and are hurtful to health and vegetation. Sulphur dioxide abounds where impure coals are consumed, and H₂S where organic decomposition takes place. It is stated that 0.06 per cent. H₂S in an atmosphere is dangerous to life, and fatal accidents in sewers have been attributed to this gas.

SO₂ may be estimated by aspirating a large and known volume of air through bromine water, and precipitating the H₂SO₄ thus formed with BaCl₂. From the weight of the insoluble BaSO₄ obtained the weight of SO₂ is calculated.

Sulphuretted Hydrogen may be detected by exposing to the air

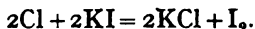
strips of filter-paper moistened with lead acetate, and estimated quantitatively by aspirating a known volume of air through a solution of decinormal iodine containing a little starch paste. Immediately the blue colour departs the aspiration is stopped.

1.7 milligrammes H_2S correspond with 1 c.c. $\frac{N}{10}$ I.



Ammonium Sulphide.—The violet colour produced by the interaction of $(\text{NH}_4)_2\text{S}$ and sodium nitro-prusside may be utilized for matching a standard solution with another containing an unknown quantity of $(\text{NH}_4)_2\text{S}$.

Chlorine may be absorbed in 10 per cent. KI solution, and the liberated I estimated with $\frac{N}{10}$ sodium thiosulphate. **Bromine** may be estimated in the same way:



3.5 parts by weight Cl = 12.6 parts I, or 7.9 parts bromine.

Nitrous, Nitric, and Hydrochloric Acids may be estimated by absorption of measured quantities of air in water, and employing the methods described in water analysis.

Carbon Disulphide.—The vapour of CS_2 found in the air of india-rubber works is estimated by passing it into strong alcoholic potash. This solution is then acidified with acetic acid, and finally neutralized with CaCO_3 . It is now diluted to twice its volume with water and titrated with standard iodine solution (1.66 milligrammes I per litre) and starch paste. One c.c. I = 1 milligramme CS_2 . The reaction is complete when a faint blue tint appears.

Chlorine and bromine are injurious to human beings in dilution of 0.1 part per 100,000, and the following as noted:

| | | | | | | |
|--|---|---|---|---|---|-------------------------|
| Iodine | - | - | - | - | - | 0.5 part per 100,000. |
| SO_2 and HCl | - | - | - | - | - | 1.0 " " |
| H_2S and NH_3 | - | - | - | - | - | 10.0 parts per 100,000. |
| CO | - | - | - | - | - | 20.0 " " |

Ozone.—Ozone, an allotropic modification of oxygen, O_3 , is a gas possessing an odour of phosphorus and an irritating action on the cells of the respiratory and conjunctival mucous membranes. It is produced by electric discharges over the sea, and to a greater extent at night than in the day. It is stated that more ozone is found in

the winter (especially after snowstorms) than in the summer. It is absent from the air of towns, living-rooms, and foggy atmospheres.

Detection and Estimation of Ozone.—Pieces of blotting-paper are soaked in a solution of KI and starch, and dried. These are then suspended in a cage, which protects them from direct sunlight, dust, and rain for twelve or twenty-four hours; where ozone is present, it liberates I, which forms a blue colour with the starch. $O_3 + 2KI + H_2O = 2KOH + I_2 + O_2$. It should be remembered that N_2O_3 , H_2O_2 , and Cl act in the same way; that free iodine may be partially volatilized, or in part form iodide or iodate of potassium, instead of blue iodide of starch; and that constant results cannot be expected owing to the variability in the conditions of temperature, light, and moisture.

Houzeau's test consists in moistening faintly red litmus-papers with a solution of KI and exposing them to the air. If ozone be present I is liberated, and alkaline KOH is formed, which renders the paper blue. Ammonia and hydrogen peroxide are the only other two gases which could produce this result. As H_2O_2 is practically never present, NH_3 is the only other gas to be considered. If, therefore, a second piece of litmus-paper untreated by KI is exposed at the same time, and if the entire colour is not due to NH_3 , the difference in the shades of the two papers must be furnished by ozone.

The intensity of colour created by ozone acting on papers exposed to the atmosphere may be matched by one of a series of ten papers forming a standard scale. Each pair of papers is exposed to a known quantity of ozone. Measured quantities of air are aspirated over the papers in tubes. If the papers are suspended in the atmosphere, wind currents, etc., by bringing unequal quantities of air into contact with them, will vitiate the results.

Hydrogen Peroxide.—Aspirate 20 to 100 litres of air containing H_2O_2 through 100 c.c. distilled water. To 10 c.c. of the water add 1 drop of a 1 per cent. potassium chromate solution, 2 or 3 drops of 25 per cent. H_2SO_4 , and 2 c.c. of ether. Shake gently for some time; perchromic acid is formed and goes into solution in the ether, rendering it blue.

Phosphoretted Hydrogen.—When grades of ferro-silicon rich in silicon (40 to 60 per cent.) are exposed to water or damp air, a

reaction takes place between calcium phosphide, Ca_3P_2 , an impurity, and the water, resulting in the production of PH_3 , and sometimes AsH_3 .

The presence of PH_3 is detected by aspirating the air containing it over two sets of filter papers—(a) moistened with a solution of AgNO_3 , and (b) moistened with a solution of $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$. The nitrate of silver only is darkened, no action taking place between the gas and lead acetate. As H_2S darkens both papers, it is well to remove it before testing for PH_3 ; this can be readily accomplished by aspirating the air through a solution of lead acetate.

Suspended Matter in the Air.—Solid animal, vegetable, and mineral particles float in the atmosphere, and tend to settle on objects as favourable conditions occur. In factories and workshops the amount of such matter may be so great as to be positively dangerous to health. Pathogenic micro-organisms adhere to dust and are carried with it.

The collection and microscopical examination of dust is effected by aspirating large quantities of air over gelatin, etc., or through water. In the first case the microscope will detect mineral and dead organic matter, and where living bacteria are present these will grow and produce colonies, which can later be subcultured and studied at length. In the second a few drops of the water are evaporated on a slide and the sediment microscopically studied.

Pouchet's aëroscope is a simple instrument in which known volumes of air are aspirated over a drop of glycerin on a microscopic slide; the intercepted particles are afterwards studied microscopically.

The dust in the atmosphere of towns commonly exceeds 10 milligrammes per cubic metre, or from 10,000 to 200,000 particles per c.c. On the top of a lofty mountain there may be no dust, or only a few particles per c.c.

As to the nature of the particles forming dust, it is sufficient to say that they are derived from every conceivable substance with which we have to do that is capable of existing in particulate form. The most important substances, from a health point of view, are solid particles capable of irritating the various internal channels in man, and bacteria and their spores.

Sewer air contains less oxygen and more CO_2 than that of the

atmosphere. Ammonia, ammonium sulphide, and various compound ammonias emitting foetid odours, sulphuretted hydrogen, and marsh-gas, are present in ever-varying quantities. Ground air is very rich in CO_2 , especially in the autumn season of the year. Ground air should be excluded from all living-rooms, not only because of its own impurity, but because where it is allowed entrance, other more dangerous gases, such as coal-gas, sewer-gas, etc., may often enter too.

A sample of ground air may be collected for examination thus: A hollow, sharp-pointed steel cylinder, with many perforations, is pushed into the soil for a distance of 4 to 6 feet. The upper end of the cylinder is connected with an air-jar, and this in turn with an aspirator. The jar being shut off from the cylinder, is first emptied by the aspirator; connection is then made, and the sample collected.

Besides CO_2 , which may reach 5 or 6 per cent., small quantities of NH_3 , CH_4 , H_2S are usually found.

Qualitative Examination of Air for Noxious Gases in Large Amounts.

Where the air of factories, etc., contains noxious gases, qualitative examination is readily performed by aspirating large quantities of the air through pure water or other suitable solvent. Where, however, the gases are in considerable quantities, tests may be applied direct to samples of the air in jars. Occasionally the atmosphere surrounding chemical works, etc., contains such large quantities of Cl , HCl , SO_2 , etc., that this direct method of examination may be adopted.

The following gases may be readily recognised by a few simple chemical tests:

HCl , CO_2 , N_2O_3 , HNO_3 , H_2S , SO_2 , Cl , CO , CS_2 , NH_3 , $(\text{NH}_4)_2\text{S}$.

1. Having collected a sample in an air-jar, remove the stopper and smell the gas. Replace the stopper quickly. Cl has a characteristic odour. HCl has a faint odour of chlorine. SO_2 has a characteristic odour, so also have NH_3 , $(\text{NH}_4)_2\text{S}$, H_2S , CS_2 .

CO_2 , CO , N_2O_3 , HNO_3 have no odours.

2. Take the reaction by moistening a red and blue filter-paper with water and rapidly inserting them in the jar, fixing the ends between the neck and the stopper. If doubt exist as to the effect on the litmus-papers, the reaction may again be taken when the gas is dissolved in a small quantity of distilled water.

HCl, CO_2 , N_2O_3 , HNO_3 , SO_2 are acid.

NH_3 , $(\text{NH}_4)_2\text{S}$ are alkaline.

H_2S , CO , CS_2 are neutral.

Cl first reddens blue litmus-paper and afterwards bleaches it.

3. Dissolve the gas in 10 c.c. of water by vigorous shaking, and if the reaction be acid, to 2 or 3 c.c. of the solution add a drop or two of AgNO_3 solution. A white precipitate indicates

(a) HCl. Acidity marked; precipitate marked and soluble in $(\text{NH}_4)\text{HO}$; insoluble in HNO_3 .

(b) CO_2 . Acidity slight; precipitate slight. Addition of $\text{Ba}(\text{OH})_2$ produces turbidity, increased on further addition of a drop or two of $(\text{NH}_4)\text{HO}$.

(c) SO_2 . Odour characteristic; acidity marked; precipitate marked, soluble in HNO_3 . Two or three c.c. of the solution from the jar added to iodide of starch will decolourize it. If 2 or 3 c.c. of the same solution be heated with a drop of HCl, a granule of $\text{Zn.H}_2\text{S}$ will be formed, which will darken lead acetate paper.

(d) No precipitate, HNO_3 . Perform the brucine test; also the diphenylamine test.

(e) No precipitate, N_2O_3 (now HNO_2). Test for nitrous acid with KI, starch, and H_2SO_4 ; and perform the meta-phenylene-diamine test.

4. If the reaction be alkaline, the gas is either

(a) NH_3 . Odour characteristic. To 2 or 3 c.c. of the solution from the jar add a drop or two of Nessler's reagent, and the well-known yellow colour is developed. Or

(b) $(\text{NH}_4)_2\text{S}$. Odour characteristic. Nessler's reagent causes a black colour when mixed with the solution from the jar. To a few c.c. add a drop or two of sodium nitro-prusside, and a violet colour rapidly appears.

5. If the reaction is neutral, one or other of the following is present:

(a) H_2S . Odour characteristic. Lead acetate paper is darkened. Solutions of salts of iron, lead, and copper produce the dark-coloured sulphides of these metals.

- (b) CS_2 . A liquid at ordinary temperatures. Set alight a drop on a porcelain slab, and note the yellow deposit of sulphur left behind.

6. The only gas which first reddens blue litmus-paper and then slowly bleaches it is Cl .

Odour characteristic. Suspend a moist KI paper in the jar. Free I will be liberated and darken the paper; later the darkened paper will be bleached. Chlorine added to a mixture of ferrous sulphate and potassium sulphocyanide produces a red colour.

Note the differences between H_2S and $(\text{NH}_4)_2\text{S}$. H_2S has a neutral reaction, odour of rotten eggs only, and forms no colour with nitro-prusside of sodium. $(\text{NH}_4)_2\text{S}$ has an alkaline reaction, odour of rotten eggs and NH_3 , and produces a violet colour with sodium nitro-prusside.

7. CO is distinguished by absence of odour, no reaction with litmus, and by the characteristic colour and spectrum when shaken with blood.

Bacteriology of the Air.—The number of micro-organisms in the air is largely determined by the quantity of dust particles in it. Bacteria adhere to and are carried by dust particles; the types found in air are for the most part chromogenic saprophytes, yeasts, and spores of moulds. The number varies with the altitude, date, and amount of recent rains, and other factors. Numerical determination is of service as a means of comparing methods of ventilation. Gordon, in his report on the ventilation of the House of Commons, 1906, states that in the dust of the chamber there were present per gramme: Streptococci, 10 to 1,000; *B. enteritidis sporogenes*, 1,000 to 10,000; *B. coli*, 1,000 to 10,000; total number of bacteria, 100,000 to 1,000,000.

Haldane found the number of bacteria in the air of book-binding workshops per litre 6, cloth factories 11, tailoring workshops 12, ropemaking premises 327.

Andrewes has shown in his reports to the Local Government Board that in certain circumstances characteristic sewage bacteria are found in the air of drains and sewers. He has carefully studied the characters of the organisms found in drain and sewer air: *B. coli* of drain air corresponds in characters with the same organism as

found in sewage. He considers that splashing produces droplets so minute as to be carried some distance in the air, and that through these bacteria are conveyed. He concludes that the number of faecal bacteria in drain air is largely proportional to the faecal content of the sewage.

The original methods of enumerating bacteria in the air designed by Pasteur, Koch, and Hesse are not in use to-day. Modern methods are of two types: (1) Those based on filtration; (2) those based on bubbling air through a suitable liquid. It is well to sow both agar and gelatin plates, as in most instances gelatin liquefies in a short time. Some form of aspirator is used, and where the air passes through a liquid, care must be taken that the air passes slowly and regularly, in order that the bubbles may burst one by one.

1. *Filtration Methods*.—Petri used a sterile wide tube containing alternate segments of wire gauze and fine sand. When aspiration was complete, the sand was mixed with sterile gelatin, and plates poured. Frankland substituted for sand glass-wool or asbestos. After aspiration the filtering medium was shaken up with broth, and with this gelatin plates were sown. But these insoluble filtering media are now displaced by soluble media.

Sodium sulphate is fused, powdered, sifted, and introduced into a glass tube, one end of which is drawn out and sealed in the flame, and the other end plugged with wool. The whole is sterilized in the hot-air sterilizer. When about to be used, the pointed end is broken off, and the other plugged and connected to an aspirator. When aspiration is finished the powdered sulphate is dissolved in a measured volume of broth, and plates are sown with known quantities of the liquid.

A mixture of glass-wool and one-third its weight of cane-sugar is used as a filtering medium in much the same manner.

2. *Bubbling Methods*.—Miguel used a Pasteur flask with two side tubulures—one drawn out and sealed, the other plugged with wool. A small measured quantity of water was placed in the flask, and the whole sterilized in the autoclave. Aspiration of air through the water was slowly effected, and when a sufficient quantity had passed through, the sealed tubulure was broken, and measured quantities of the water sown in media, and the latter incubated.

Laveran uses two glass tubes connected at the junction of

their upper and middle thirds by a bridge tube. Each of the upright tubes is plugged with an india-rubber stopper carrying a pipette which reaches to the bottom of the tube. The pipettes are plugged above with wool. One pipette is graduated in tenths of a c.c. The tube carrying the other pipette has a 10 c.c. mark on the glass. Ten c.c. of a 1 per cent. solution of sugar in water are placed in this tube, and the apparatus is autoclaved.

When about to use, remove the plug from the pipette which dips in the sugar solution, and connect the other pipette with an aspirator. The aspirated air bubbles through the solution, into the first tube, through the horizontal connecting-tube, down through the second tube, and passes out through the pipette connected with the aspirator. When sufficient air has bubbled through, gently aspirate the sugar solution into the entry pipette to wash it; then run the liquid through the connecting-tube into the second upright tube, and so into the second and graduated pipette; repeat this several times so as to collect all the bacteria that have been caught on the glass. Now, by the graduated pipette, distribute the liquid into the various culture media.

This method is suitable for large volumes of air, and supplies plenty of material for sowing cultures. It is thus one of the best methods for detecting pathogenic bacteria.

Suppose 250 litres have been aspirated and 20 colonies have grown on a gelatin plate sown with 1 c.c. of the sugar solution:

$$20 \times 10 \times \frac{1000}{250} = 800 = \text{number of aerobic organisms contained in a cubic metre of air (1,000 litres = 1 cubic metre).}$$

A simple method which may be made by careful manipulation fairly accurate is that of plate exposure: Pour Petri plates of gelatin and agar. When solid, expose them to the air under examination by removing their covers for selected periods—say fifteen to thirty minutes. At the end of the period replace covers and incubate. When organisms have developed, count and calculate to units of area and time—say per square foot per minute (area of a Petri dish = πr^2 where r is the radius). If necessary, the various subcultural methods may be resorted to for the identification of individual species.

CHAPTER XI

FOODSTUFFS

MILK.

SINCE the milk of the cow is used to a much greater extent than that of any other mammal, its composition and properties have been much more thoroughly studied. Its liability to early decomposition and the fact that it forms an excellent culture medium for bacteria render it necessary that the strictest attention should be paid to its production, collection, and distribution:

Composition of cow's milk:

| | | | | Per Cent. |
|----------|----|----|----|-----------|
| Water | .. | .. | .. | 87.75 |
| Proteins | .. | .. | .. | 3.50 |
| Lactose | .. | .. | .. | 4.60 |
| Fat | .. | .. | .. | 3.40 |
| Ash | .. | .. | .. | 0.75 |

Our knowledge of the proteins of milk is still very incomplete. The application of ordinary and crude chemical methods to the investigation of vital products necessarily leads to unsatisfactory results. The preparation of pure proteins is a most difficult task, and the probabilities are that in many cases where it is thought that a pure product has been isolated it is contaminated by reagents.

The proteins of the milks of different animals vary considerably. On the addition of an acid to cow's milk or goat's milk, a curd or clot composed of casein is formed, and it is believed that in these cases the casein is chemically combined with the phosphates of the alkaline earths. In human milk and the milk of the ass and mare no such clot is produced on the addition of acid. Here it is believed that the protein is not combined with phosphates. Besides casein, a second protein (lactalbumin) is found in all milks. Storch de-

scribes a muco-protein which he holds forms a gelatinous envelope round the fat globules. Amylolytic and proteolytic ferments are said to occur in milk.

The protein molecule is highly complex, as evidenced by its indiffusibility. Through the action of enzymes in the presence of acids and alkalies these complex bodies are hydrolysed, passing through various intermediate stages (varieties of albumoses) into diffusible peptones. Further hydrolysis produces amino-acids.

The number of proteins in the milk of the cow has been variously stated. Duclaux maintains that there is only one—casein, existing in two forms, coagulable casein and non-coagulable casein. Hammarsten describes two—casein, corresponding to Duclaux's coagulable casein, and lactalbumin, corresponding to Duclaux's non-coagulable casein. This observer admits that lactalbumin has the properties of a true albumin, and closely resembles serum albumin; but holds that, owing to differences in certain physical constants, it is a distinct body. Hammarsten's casein and Halliburton's caseinogen are doubtless the same body. Sebelein describes a globulin in milk.

Casein.—When pure, this is a white, non-crystalline, odourless, and tasteless substance, insoluble in water, weak acids, alcohol, and ether. It is soluble in stronger acids and weak alkalies. It appears to possess a peculiar affinity for calcium phosphate as it is almost, if not quite, impossible to free it from this salt. Casein contains less sulphur than either globulin or albumin, but much more phosphorus. In solution in weak alkalies it is lævo-rotatory on polarized light. Béchamp holds that it is a weak dibasic acid, forming two types of salts, and his view is confirmed by Söldner. This body is readily prepared by diluting milk about five times and adding acetic acid until the solution contains 0.1 per cent. The precipitate formed carries down the fat with it. This precipitate is well washed on a filter, dried by pressure, and dissolved in the least excess of ammonia. By this means the fat rises to the surface and the underlying solution can be siphoned off. It is again precipitated by acetic acid, washed, dried, and redissolved in ammonia. After three or four such precipitations the casein is rubbed up with alcohol in a mortar. The alcohol is poured off, and the residue treated in the same manner with ether. It is afterwards extracted

with ether in a Soxhlet apparatus to remove the fat. The treatment with alcohol and ether is repeated a number of times. It is finally dried at 100° C. If dried whilst containing water, it forms a hard horny mass.

Söldner showed that two lime compounds exist (CaO and 2CaO) to one molecule of casein ($\text{C}_{170}\text{H}_{268}\text{N}_{48}\text{SPO}_{51}$).

Lactalbumin.—This protein coagulates at 70° C., although the precipitation is never complete. Like other albumins it is not precipitated by saturating its solutions with MgSO_4 . It is precipitated like other albumins, by saturating its solution with Na_2SO_4 . Its rotatory power is $[\alpha]_D = -67.5^\circ$.

It can be prepared by saturating milk with MgSO_4 , filtering, and adding to the filtrate acetic acid until 0.25 per cent. of the solution is reached, when lactalbumin is precipitated. It is redissolved in water, again saturated with MgSO_4 , and reprecipitated with the same strength of acetic acid. This treatment is repeated three or four times. The solution of lactalbumin is next dialyzed to remove salts. When the salts have been got rid of, the solution is precipitated with alcohol and ether, and dried at a low temperature. The result is a tasteless white powder completely soluble in water.

Lactoglobulin.—This protein is not coagulated by rennet, but is coagulated by heat and neutral sulphates. It occurs in very small quantities in milk, and it is doubtful whether it is distinct from serum globulin.

Muco-Protein of Storch.—This body is insoluble in dilute ammonia and weak hydrochloric acid. It is partially soluble in the hydroxides of potassium and sodium, undergoing at the time of mixture considerable increase in bulk. It gives the characteristic protein reactions with the xantho-proteic and Millon's tests. On gently heating with dilute sulphuric acid it yields a reducing sugar. When washed with alcohol and ether and dried at the ordinary temperature of the atmosphere it forms a light greyish powder which is very hygroscopic.

It may be prepared by centrifugalizing separated milk and washing the deposit with weak ammonia-water. The resulting mass is then well washed with alcohol and ether, and dried. Or it may be prepared from cream. Storch has, by means of benzene, alcohol, and ether, separated it from butter.

Milk contains traces of extractives and colouring matters. Its characteristic white appearance is held to be due to the interference of light rays produced by casein in pseudo-solution, a state in which particles exist in the solution not of sufficient size to settle under gravity, but which interfere with the passage of light. These particles can be separated by a current of electricity. There is no sharp line of division between crystalloids and colloids in solution, substances in pseudo-solution, and bodies in suspension. In milk, fat is in suspension, casein in pseudo-solution, albumin in solution as a colloid, and lactose in solution as a crystalloid. The variety in size of the particles or masses of molecules probably determines the presence of one or other of these states in a given case.

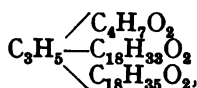
Lactose.—Lactose ($C_{12}H_{22}O_{11} \cdot H_2O$) is an aldose, and exhibits the constitution of a galactose-glucoside in that on hydrolysis by acids it produces a mixture of galactose and glucose. The aldehyde group of the galactose has been eliminated in lactose, whilst the glucose remains.

Several modifications of milk-sugar are known, distinguishable from each other chiefly by their action on polarized light. Lactose, like other aldoses and ketoses, reduces alkaline solutions of $CuSO_4$, forming cuprous oxide, the well-known Fehling's reaction. Each sugar effects a definite amount of reduction, and this affords an excellent method of distinguishing them. Lactose differs from other sugars in that its osazone forms an anhydride soluble in boiling water.

Lactose is hydrolysed by a specific enzyme lactase found in certain torulæ, in some kefir preparations, and in aqueous extract of almonds. Lactose is not hydrolysed by maltase, invertase, or diastase. It easily undergoes lactic and butyric acid fermentations. Mineral acids hydrolyse it to glucose and galactose. It reduces Fehling's solution, and exhibits mutarotation. It is manufactured by evaporation of whey, the resulting crystals being purified by recrystallization.

Fat of Milk.—The fat of milk consists of a mixture of ethereal salts of glycerol, forming small globules ranging in size from 0.001 millimetre to 0.01 millimetre.

It is highly probable that there are three separate acid radicles combined with each glycerol group, thus:



a compound of the acid radicles of butyrim, olein, and stearin with glyceryl.

Milk-fat has the following composition:

| | | | | Per Cent. |
|----------|----|----|----|-----------|
| Butyrim | .. | .. | .. | 3.90 |
| Caproin | .. | .. | .. | 3.45 |
| Caprylin | .. | .. | .. | 0.50 |
| Caprin | .. | .. | .. | 1.85 |
| Myristin | .. | .. | .. | 20.30 |
| Laurin | .. | .. | .. | 7.50 |
| Stearin | .. | .. | .. | 2.00 |
| Palmitin | .. | .. | .. | 25.50 |
| Olein | .. | .. | .. | 35.00 |

In addition to the above fats, traces of certain extractives, such as urea, lecithin, cholesterin, together with colouring matters, exist in the fat of milk.

The vexed question of the presence or absence of a definite membrane round the fat globule will not be discussed in this work. It may be stated in a word that Béchamp from his studies of the appearances found on mixing ether with milk and of the behaviour of milk towards certain stains has concluded that an endosmotic membrane exists; whilst Storch by his observations is led to believe that instead of a definite membrane a muco-protein capsule encloses the fat globule and insensibly shades off into the surrounding fluid.

Human milk has the following composition:

| | | | | Per Cent. |
|---------|----|----|----|-----------|
| Water | .. | .. | .. | 88.2 |
| Fat | .. | .. | .. | 3.3 |
| Casein | .. | .. | .. | 1.0 |
| Albumin | .. | .. | .. | 0.5 |
| Lactose | .. | .. | .. | 6.8 |
| Ash | .. | .. | .. | 0.2 |

The fat globules are smaller than those of cow's milk, ranging from 0.009 to 0.0009 millimetre. Its composition varies much more than that of cow's milk. It contains small quantities of citric acid. It is almost always alkaline.

When milk is allowed to stand for a time, a series of well-known

changes succeed each other. The fat, the lightest portion, rises to the surface as cream. After a variable period, depending on the temperature, presence of certain micro-organisms, and other factors, the milk becomes acid and separates into solid curd and liquid whey. The principal agent in this reaction is the *B. lacticus*, which converts lactose into lactic acid. Other micro-organisms, such as the *B. butyricus*, *B. coli communis*, etc., are also capable of forming acid, and thereby curdling milk. Rennet is used artificially for bringing about the same change. The curd consists of precipitated proteins with entangled fat, and the whey of water, lactose, and salts. The cream of ordinary milk forms about 10 per cent. by volume of the whole.

The variations in the composition of milk, even from the same animal, are due to a number of factors, such as the health of the animal, the age—young animals secrete less milk and a product of poorer quality—the time that has elapsed from the last milking, the stage of milking, the breed of the animal, the time that has elapsed since previous parturition, the nature of the food eaten, etc.

There are, however, limits to these variations, and all good milks at all times fall within these limits. Fatty solids may range from 2 to 7 per cent., non-fatty solids from 8 to 11 per cent., ash from 0.6 to 0.9 per cent., cream from 2 to 25 per cent., specific gravity from 1.027 to 1.037.

But it is rare that the fatty solids fall below 3 per cent., and the non-fatty solids below 8.5 per cent., and these figures are insisted upon by law.

Comparative analyses of various milks are represented in the following table:

| | | | | Water. | Proteins. | Fat. | Lactose. | Ash. |
|---------------|----|----|----|--------|-----------|------|----------|------|
| Cow | .. | .. | .. | 87.7 | 3.5 | 3.4 | 4.6 | 0.7 |
| Human subject | .. | .. | .. | 88.2 | 1.5 | 3.3 | 6.8 | 0.2 |
| Goat | .. | .. | .. | 86.0 | 4.3 | 4.6 | 4.2 | 0.7 |
| Mare | .. | .. | .. | 89.8 | 1.8 | 1.1 | 6.9 | 0.3 |
| Ass .. | .. | .. | .. | 90.1 | 1.6 | 1.2 | 6.5 | 0.4 |
| Ewe | .. | .. | .. | 79.4 | 6.7 | 8.6 | 4.3 | 0.9 |

In cattle-plague and foot-and-mouth disease marked changes occur in the milk of the animals affected. The quantity is diminished, the curd separates out quickly on heating from a pale blue whey, and blood and pus corpuscles are generally present. In

tuberculosis the milk is not markedly affected except in those rare cases in which the udder is extensively diseased.

The Analysis of Milk.

A chemical analysis is of service from a public health point of view in detecting the removal of fat, the addition of water, or both, and the presence of artificial colouring matters and preservatives. A bacteriological examination is often necessary in the investigation of milk-borne epidemics—such as enteric, diphtheria, etc.—and for the detection of tubercle bacilli; to obtain evidence respecting the healthiness of the udder, etc., of the cow; and to measure the general bacterial content, and especially the degree of contamination from faecal matter.

For chemical analysis the milk must be fresh, as after standing for a time the lactose is transformed into lactic acid, and the non-fatty solids consequently diminished.

Reaction.—The reaction is mostly alkaline, sometimes amphoteric when litmus is used as an indicator. This is due to the presence of NaH_2PO_4 and Na_2HPO_4 , the first turning blue litmus red and the second red litmus blue.

Estimation of Total Acidity—Lactic Acid.—Place 100 c.c. of the milk in a beaker, add 5 c.c. of a 0.1 per cent. phenolphthalein solution, and titrate with $\frac{N}{10}$ NaOH until a faint pink tint appears.

It will be found that generally 20 c.c. of $\frac{N}{10}$ NaOH is required; each c.c. of the decinormal alkali represents 1 degree of acidity.

If litmus be used as indicator instead of phenolphthalein, a smaller figure will be obtained, as the salts of milk and carbonic acid are not sensibly acid to litmus-paper; for this reason litmus may be used in roughly determining the quantity of lactic acid. There is no good method for quantitatively estimating lactic acid. When milk is boiled, its acidity is diminished.

Specific Gravity.—Specific gravity is the weight of unit volume, and may be determined in two ways: first, by finding the weight of a known volume, and second, by finding the volume of a known weight. The first method may be used by taking the weight of liquid which fills a vessel of known volume—example, specific-gravity bottle, or Sprengel's tube. This method may also be used

by immersing a plummet of known volume in the liquid, and noting the loss of weight due to the displacement of the same volume of liquid—example, Westphal's balance. The second method is applied by immersing a float of known weight in the liquid, and noting the volume immersed, which will be equal to a volume of the liquid of the same weight as that of the float—example, hydrometers, of which the lactometer is a special form used in testing milk.

In using the specific-gravity bottle, which is perhaps the most exact method, care should be taken that the bottle is clean. It is well to observe the ritual of subjecting the bottle to cleansing with weak acid, water, alcohol, and ether on each occasion before use, and to weigh it direct from a desiccator. The bottle is first weighed. It is then filled with milk, the stopper is gently let in, and its hollow channel is filled to the top with the fluid. Any superfluous milk is carefully wiped away with a clean and dry duster, and the bottle is again deposited in the desiccator for a short period before weighing a second time. The temperature should remain constant and at 15.5°C . during the entire process.

The second weight minus the first is equal to the weight of the milk contained in the bottle. This weight divided by the weight of the same volume of distilled water at the same temperature is the specific gravity. Most specific-gravity bottles have the weight of distilled water which they contain at 15.5°C . marked on their surface, so that it is unnecessary to take this weight.

Taking the specific gravity of H_2O at 15.5°C . as 1.000, that of milk is about 1.032. It is obvious that the removal of fat which is the lightest constituent of milk raises the specific gravity, and its addition lowers it. The addition of water also lowers the specific gravity. So therefore a low specific gravity may mean either abundant fat or added water.

The specific gravity of milk is observed to rise slightly for some hours after milking—*e.g.*, a milk of specific gravity 1.031 when drawn from the cow may in ten hours show a specific gravity of 1.032; this rise is known as 'Recknagel's phenomenon.'

If the quantity of cream as measured in a cream-tube reading percentages be the normal 10 per cent. after standing twenty-four hours, and the specific gravity be found low, it is clear that water has been added.

The Westphal balance consists of a graduated swinging arm resting on a knife-edge and a glass plummet suspended from a hook attached to one end of the arm. The other end of the arm is drawn out to a point which when the balance is adjusted and the plummet hangs in air should rest exactly opposite a similar point on the frame. Three riders are used on the graduated arm: their weights are wholly empirical, and indicate hundreds, tens, and units respectively.

The milk or other fluid is poured into a glass cylinder; the arm is raised or lowered by means of a screw in the upright support until the plummet is just completely reversed, and the riders are so placed on various divisions of the scale that the points come to rest exactly opposite each other. Supposing that in an estimation the largest (as must be) is suspended from the hook carrying the plummet, the tenth division of the scale, and the tens and units riders rest on the scale divisions 3 and 2 respectively, when the point of the swinging arm comes to rest at zero the specific gravity will be $100 \times 10 + 10 \times 3 + 1 \times 2 = 1.032$.

Lactometers, special forms of hydrometers, are less accurate in estimating specific gravities.

The specific gravity of milk varies between 1.013 and 1.039.

By removal of all the cream from a milk of specific gravity 1.032, the figure is raised to 1.036. On the other hand, by adding 4 per cent. fat to the same milk, the specific gravity is reduced to 1.028. The specific gravity test is not an absolute one, but a useful preliminary test. Like most substances, milk alters in specific gravity with change of temperature. It does not share, however, the peculiarity which water possesses of attaining its maximum specific gravity at 4° C. It decreases in specific gravity from freezing-point (−0.5° C.) upwards. Tables of corrections for temperature have been constructed when the determination is made at temperatures above or below 15.5° C.; but it will be sufficiently exact to add or subtract 1 degree of specific gravity for every 6 degrees of temperature registered above or below 15.5° C.

The Fat.—Of the many methods at present in use for the estimation of fat, the following two are to be recommended, and the first is preferable to the second:

1. ADAMS'S PROCESS.—In this gravimetric method the solvent

used for the extraction of fat is ether, convenient on account of its low boiling-point and heat of volatilization, its high solvent power for fat, and its miscibility with water.

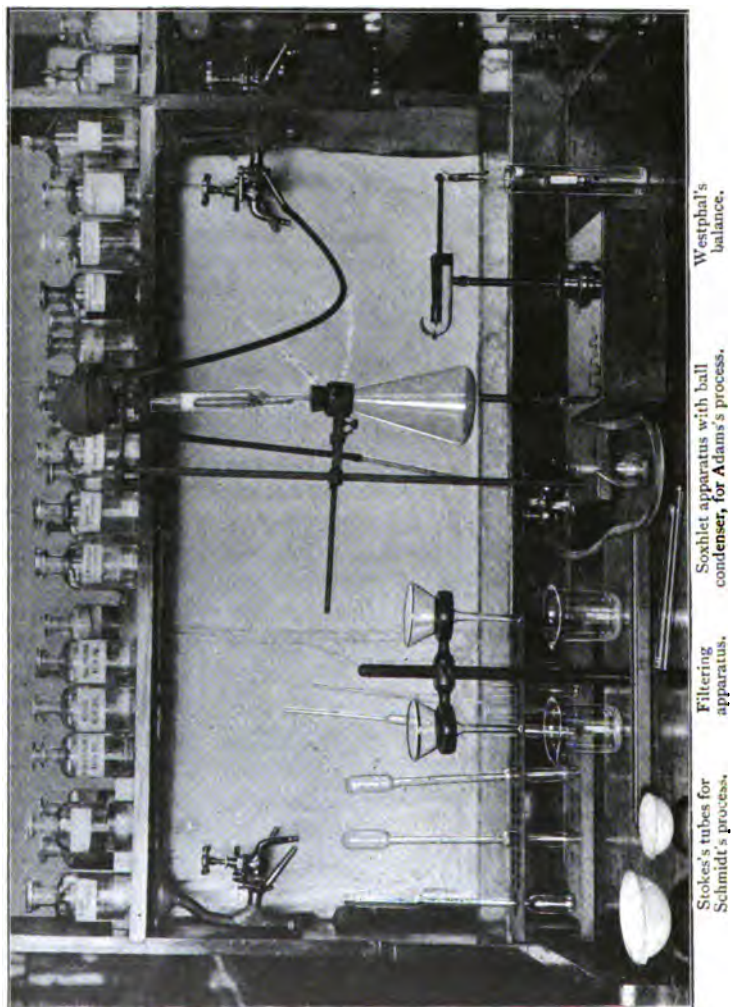


FIG. 26.

When milk is dropped on blotting-paper, it spreads out to a much greater degree than when placed on glass or in a dish, and Adam

considered that extraction of the fat by ether would accordingly be much more complete. After passing through various stages of evolution, the process is now carried out somewhat as follows:

A strip of Schleicher and Schüll's fat-free paper is hung up by one end. The other end is held in the fingers so that the surface of the strip is as nearly as possible horizontal, and 5 c.c. of the sample of milk carefully measured in a pipette are distributed over the paper. The weight of this volume is determined by running into a convenient weighing vessel 5 c.c. of the same sample at the same rate as it was run on to the paper. The paper is allowed to hang until dry, and must be protected from flies and all other disturbing influences. When dry the paper is rolled up into a loose coil of a diameter such that it will easily pass into the Soxhlet extractor (say $\frac{3}{4}$ inch). A blank coil containing no milk should be dried and rolled up in the same way, and both further dried at 100° C. for an hour. Each coil is then placed in a Soxhlet extractor, arranged in an upright position, and connected with vertical condensers. Small weighed flasks of 150 c.c. capacity containing dry ether in sufficient quantity to fill the extractor well above the upper portion of the siphon, are attached to the lower end of the Soxhlet apparatus, and the ether is made to boil by immersing the flask in water at 55° to 60° C. Extraction should be continued for two to three hours, although many analysts are satisfied with twelve to eighteen siphonings.

The flasks containing ether and dissolved fat are then disconnected, the ether is driven off by evaporation, and the flasks dried and weighed.

The difference in weight represents the fat; the small amount of extract derived from the paper of the blank experiment is finally subtracted from the weight of fat found for the sample, and the difference represents the fat contained in 5 c.c. The weight of 5 c.c. has been determined; accordingly the percentage of fat is readily calculated.

Sour milk may be operated on if the acidity be neutralized by $\frac{N}{16}$ NaOH, using litmus as indicator.

It is advisable to put a small piece of blotting-paper in the mouth of the open tube at the top of the condenser so as to limit the

entrance of moist air which would slightly wet the ether. In driving off the ether from a flask, it is well to lay the flask on its side in one of the openings of the water-bath, and afterwards, when the drying is being completed in an air oven, the flask should be rotated from time to time, and air blown in every five minutes, to remove ether vapour.

Dry ether is prepared by washing commercial ether with water, shaking the washed ether with calcium chloride, and, after allowing it to stand over calcium chloride for a day or two, distilling.



A B C D E F

FIG. 27.

A, Evaporating basin; B, specific-gravity bottle; C, fat flask; D, pipette; E, boiling-tube; F, 100 c.c. stoppered cylinder.

Sufficiently dry ether may also be obtained for most purposes by distilling the commercial variety and rejecting the first fractions which pass over below 34.3°C. , and the last above 34.8°C.

If at any time doubt exists as to the completion of the extraction process, a second weighed flask containing fresh ether should be affixed, and the process continued for some time. This flask after evaporating the ether and drying at 100°C. should not increase in weight.

2. THE WERNER-SCHMIDT METHOD.—The specific gravity of the sample is ascertained or a measured volume is weighed. Fifteen c.c.

are pipetted into a boiling-tube and a like measure of pure hydrochloric acid added. The mixture is shaken up and gently boiled until the contents appear dark brown in colour. The boiling must not be continued too far, as certain bodies soluble in ether are liable to be formed from the milk-sugar. The process is not suitable for milks containing cane-sugar. Boiling with acid renders the casein soluble, and so eliminates the obstacles which, in the solid condition, it offers to the extraction of fat.

When cold, pour the contents of the tube into a graduated and stoppered 100 c.c. cylinder. Wash out the tube with ether, and finally make the column up to 75 c.c. with ether. Invert the cylinder several times, and put aside to settle. Read the height of the ethereal column, including three-fourths of the thin grey layer of casein. Draw off an aliquot part of this column and evaporate; dry, and weigh the residual fat in a small flask, in the manner described in Adams's process.

Stokes's tube, a specially graduated tube prepared to treat 10 c.c. of milk, is also employed in this country. After completing the boiling with 10 c.c. of HCl, and cooling, ether is added until the surface of the column reaches the 50 c.c. mark. An aliquot portion of this column is afterwards drawn off, evaporated, dried, and the residue weighed as above. The special tube is not to be recommended, as the narrowed central portion offers resistance to the free escape of hot air during boiling, and the consequent explosive action frequently causes loss of the contents.

This process is much more rapid than that of Adams, and in skilled hands almost as accurate.

The student will remember that, owing to the low boiling-point of ether, it should never be added to a hot solution, and will accordingly always cool the boiling-tube before adding it. This may be rapidly done by holding it under a water-tap. In drying and weighing the fat it is essential that the last trace of ether vapour be got rid of by blowing dry air into the flask, and by ascertaining that two successive weighings, separated by half an hour's heating at 100° C., are the same.

Example.—A milk whose specific gravity is 1.032 is subjected to the Adams process, and the fat collected in a flask weighing

16.056 grammes. The weight of the flask and fat is 16.236 grammes. The weight of the fat is therefore 0.180 gramme.

5 c.c. of specific gravity 1.032 = 5.16 grammes.

If now 5.16 grammes of milk yield 0.18 gramme fat, what is the percentage of fat ?

$5.16 : 100 :: 0.18 : \text{the percentage.}$

Percentage therefore = 3.5 nearly.

The same sample subjected to the Werner-Schmidt process yielded practically the same result.

15 c.c. of the milk yielded 0.542 gramme fat;

but 15 c.c. of specific gravity 1.032 = 15.48 grammes;

$15.48 : 100 :: 0.542 : 3.5.$

3. There are several forms of centrifugal apparatus used for estimating fat, such as the Babcock, Leffmann-Beam, Gerber, etc.

The Leffmann-Beam provides small flasks graduated on the neck into eighty divisions—ten divisions corresponding to 1 per cent. of fat. Run into the flask 15 c.c. milk. Add 3 c.c. of a mixture of equal parts HCl and amyl alcohol; shake and add slowly with agitation 9 c.c. concentrated H_2SO_4 . Fill up to zero with hot mixture of equal parts concentrated H_2SO_4 and water. Place in the centrifuge and rotate for two minutes. If fat and acid liquid are both quite clear, read off the fat column; if fat or acid liquid be cloudy, rotate again. The amyl alcohol assists in the collection of the fat globules: this reagent should be good, otherwise large error may occur, generally in the direction of excess of the truth. Where the operation is carefully carried through with sound reagents results are obtained to within 0.15 per cent. of those got by the Adams's process.

The reagents used in the Gerber process are H_2SO_4 and amyl alcohol. The centrifuge runs on ball bearings, and reaches a velocity of about 2,000 revolutions per minute. Three minutes are sufficient to separate the fat.

In the Babcock method H_2SO_4 and boiling water are employed as reagents. The centrifuge revolves at about 1,000 times per

minute—hence a longer time is required for completing the separation of fat (seven to eight minutes).

These centrifugal methods are used for determining the fat—(1) in condensed milk after it has been diluted to form a 10 per cent. solution; (2) in cream after it has been diluted five or six times with hot water; (3) in butter and (4) in cheese after mixture with a small quantity of cold or hot water, as suitable, by addition of modified quantities of the reagents, and the necessary rotation.

Estimation of Cream.—A cream-tube or creamometer provided with a short scale at its upper part, each division of which reads 1 per cent. of the capacity of the tube up to the highest line (zero), is filled to the zero with the milk to be tested, and set aside for six, twelve, or twenty-four hours, and the volume of cream measured. A good milk should throw up 10 per cent. of cream in eight hours. The method is by no means accurate, as the same milk under different conditions of setting may show very marked differences in the quantity of cream formed.

The estimation of fat in dried milk may be made by the Werner-Schmidt method or by the Röse-Gottlieb method, preferably the latter.

Röse-Gottlieb Method.—Weigh 0.5 gramme milk powder into a stoppered cylinder holding about 50 c.c. Add 5 c.c. water and 0.5 c.c. ammonia (equal parts 0.88 ammonia and water). Shake and warm if necessary until solution is obtained. Add 5 c.c. alcohol and shake again until homogeneous. Now add 12.5 c.c. ether and mix very thoroughly; finally add 12.5 c.c. petroleum ether (boiling below 60° C.) and again mix thoroughly. Settle out and draw off the ether. Repeat the extraction with a mixture of equal parts ether and petroleum ether until the whole of the fat is recovered. Remove the solvent by distillation, dry, and weigh the fat.

Total Solids.—Pipette into a clean, weighed, platinum dish 10 c.c. of the milk, and reweigh to obtain the weight of the milk used. Heat on a water-bath, breaking up occasionally the film that forms on the surface, in order to hasten evaporation. After an hour's drying, the dish is removed to a tray, carrying two or three layers of blotting-paper to remove moisture, and the tray is placed in an air oven at a temperature of 95° C., and provided

with sufficient draught; here the drying is completed. It may require two to three hours in the oven to produce a constant weight. Platinum basins are preferable to porcelain, as they cool much more rapidly, and thus require less time in the desiccator. As milk solids are highly hygroscopic, no time must be lost in conveyance from the desiccator to the balance, nor in the process of weighing.

Supposing that the specific gravity of the milk is 1.032, the weight of 10 c.c. will be 10.32 grammes. Further, supposing the difference in the first and second weighings of the dish to be 1.3 grammes, the percentage of total solids will be found from the proportion:

$$10.32 : 100 :: 1.3 : x;$$

$$x = \frac{100 \times 1.3}{10.32} = 12.5.$$

From the total solids the ash is obtained by ignition at a low heat over an argand burner. The last trace of dark, separated carbon must disappear and the residue consist of a greyish-white mass before the dish is removed from the flame. Overheating causes loss of NaCl. Cooling, weighing, and percentage calculation are carried out in the usual manner.

The P and S of the milk proteins produce phosphoric and sulphuric acids. Carbonic acid is formed by the combustion of organic carbon. The ash does not truly represent the inorganic constituents. It is computed that at least 8 per cent. of the phosphoric acid arises from the P of the casein. Bases predominate over acids in milk, and unite with proteins to form soluble protein salts, and with citric acid to form citrates.

Composition of ash:

| | | | | Per Cent. |
|-----------------|----|----|----|-----------|
| Lime | .. | .. | .. | 19.3 |
| Phosphoric acid | .. | .. | .. | 28.3 |
| Potash | .. | .. | .. | 27.7 |
| Chlorine | .. | .. | .. | 13.9 |
| Soda | .. | .. | .. | 6.7 |
| Ferric oxide | .. | .. | .. | 0.3 |
| Magnesia | .. | .. | .. | 2.7 |
| Carbonic acid | .. | .. | .. | 1.0 |
| Sulphuric acid | .. | .. | .. | 0.1 |

A probable composition for the salts as they exist in milk has been theoretically calculated:

| | Per Cent. |
|---|-----------|
| NaCl | 10.62 |
| KCl | 9.16 |
| KH_2PO_4 | 12.77 |
| K_2HPO_4 | 9.22 |
| $\text{K}_2(\text{C}_6\text{H}_5\text{O}_7)$ | 5.47 |
| MgHPO_4 | 3.71 |
| $\text{Mg}_2(\text{C}_6\text{H}_5\text{O}_7)$ | 4.05 |
| CaHPO_4 | 7.42 |
| $\text{Ca}_3(\text{PO}_4)_2$ | 8.90 |
| $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ | 23.55 |
| Lime combined with proteins | 5.13 |

If the ash is materially less than 0.73 per cent., watering may be suspected.

Solids not Fat.—This item of the analysis is calculated by finding the difference in weight between the total solids and the fat. The solids not fat have been found to vary between 5 and 10 per cent. The law fixes 8.5 as the lower limit for whole milk.

In case it is necessary to determine the percentage of proteins in milk, the best method to employ is the following modification of Kjeldahl's method for the estimation of total organic nitrogen, and multiply the result by 6.38. To obtain the total organic nitrogen, weigh 5 grammes of milk into a Kjeldahl flask of about 150 c.c. capacity, and add 20 c.c. pure H_2SO_4 . Place over a small flame in the fume-chamber, and heat till thoroughly charred. Remove the flame, and add 10 grammes bisulphate of potash to raise the boiling-point of the mixture. Place a pear-shaped bulb in the neck of the flask and apply the flame, increasing its size as frothing ceases. The liquid becomes colourless in thirty minutes or thereabouts. Cool, dilute largely with water, and transfer to the distillation-flask provided with perforated cork carrying a dropping funnel with stop-cock, and a wide tube with one or more bulbs blown in it, which are loosely packed with asbestos. One end of the tube is connected with a condenser, and the other is made to dip below the surface of 50 c.c. $\frac{\text{N}}{10} \text{H}_2\text{SO}_4$.

Through the dropping-funnel pass about 100 c.c. of a 20 per cent. solution of NaOH. Shake well by a rotatory motion. Apply a flame to the distilling-flask and collect about 200 c.c. of the distillate. Take care that the condenser remains throughout quite cold. Titrate with $\frac{\text{N}}{10} \text{NaOH}$, using litmus as indicator. Subtract

the number of c.c. $\frac{N}{10}$ NaOH solution used from the 50 c.c. $\frac{N}{10}$ sulphuric acid, and the remainder represents the acid neutralized by the ammonia distilled over. From this deduct the figure obtained in a blank experiment in which all the factors are exactly the same, except that milk is eliminated.

Each c.c. of the $\frac{N}{10}$ H_2SO_4 neutralized by ammonia is equal to 0.0014 gramme of N, which, when multiplied by 6.38, is equal to the total protein.

Colostrum is a term applied to the first milk secreted after parturition. Houdet describes two forms—a viscous, brownish product, and a non-viscous lemon-yellow liquid; the earlier milkings furnish the first, and the later the second; the two co-exist often in the same animal. The fat differs somewhat from that of ordinary milk, in that its melting-point is high (42° C.) and its Reichert-Wollny figure low (6 to 7). The most characteristic feature of colostrum is the presence of the *corps granuleux* of Donné, consisting of cells clustered together like bunches of grapes, and measuring in diameter from 0.005 to 0.025 millimetre. The specific gravity of colostrum averages 1.068.

Estimation of Citric Acid in Milk.—Prepare acid nitrate of mercury by dissolving mercury in twice its weight of HNO_3 (specific gravity 1.42), and adding an equal volume of water.

With this reagent precipitate the proteins of the milk, and filter until the filtrate is clear. Rapid clearing may be effected at this stage by addition of some super-saturated solution of aluminium hydrate. To a measured volume of the filtrate add dilute caustic soda solution until the neutral point is reached (phenolphthalein as indicator). Filter off the white precipitate of calcium phosphate, calcium citrate, and mercuric nitrate; wash well with water; remove from the filter, and suspend in water, to which a little dilute HCl has been added. Pass H_2S through the fluid until all the mercury comes down as HgS . Filter again, and boil the filtrate to remove H_2S . Add a little calcium chloride and cool. Carefully neutralize a second time with dilute caustic soda, and filter off the calcium phosphate. Concentrate the filtrate to small bulk. This contains the citric acid as calcium citrate. After thorough boiling, filtering, and washing the precipitate with boiling water, ignite it, and add to it excess $\frac{N}{10}$ HCl. Titrate back the excess

with $\frac{N}{10}$ NaOH (methyl orange as indicator). Each c.c. $\frac{N}{10}$ HC used = 0.007 gramme citric acid.

Action of Heat on Milk.—When heated to 70° C., the albumin of milk, although not precipitated, is so changed that it is readily precipitated by acids and $MgSO_4$. At 80° C. a further unknown change occurs in certain organic constituents, recognizable by the fact that they cease to evolve a gas from H_2O_2 , and to produce a blue colour with paraphenylenediamine and H_2O_2 .

Test for Boiled Milk.—Shake 5 c.c. milk in a test-tube with 1 drop of a 2 per cent. H_2O_2 solution and 2 drops of a 2 per cent. paraphenylenediamine solution. If the milk has not been heated above 80° C., a dark violet or blue colour appears at once; but if it has been boiled or pasteurized to this temperature, no colour appears. At 100° C. calcium citrate is deposited, and a reduction in the rotatory power of milk-sugar takes place.

When milk is heated in contact with air a protein film, probably an oxidation product, is formed on the surface, which has been variously classified.

It has been claimed that boiled milk is more easy of digestion than the raw secretion, and perhaps this is true. The much-discussed question of boiled milk producing symptoms of scurvy has not been settled; there is no reliable evidence from which a conclusion may be drawn.

Lactose.—An important product of milk in connection with the artificial feeding of infants is milk-sugar. Its estimation may be carried out as follows:

To 50 c.c. distilled water add 6 grammes of milk-sugar finely powdered, and stir with a thermometer for ten seconds; allow to settle for twenty seconds, and read the fall in temperature. Filter, and fill a polariscope tube with the clear filtrate. Take readings every minute until the specific rotatory power begins to diminish. When the polarized solution is kept at a temperature of 15° C., several readings can be obtained which are nearly constant; the mean of these is the *initial* rotation. After twenty-four hours, polarize again at the same temperature to obtain the *normal* rotation. The $\frac{\text{initial rotation}}{\text{normal rotation}}$ is the 'birotation ratio.'

The amount of sugar may be estimated in 100 c.c. of the above

solution by dividing the normal rotation reading in angular degrees by 1.106.

Principles of Polarimetry.—The vibrations which constitute an ordinary light ray take place in all directions in a plane perpendicular to the line of propagation of the ray. If one looks at an object through a crystal of Iceland spar, two images are seen—the light ray has been split by the crystal into two, the more refracted or *ordinary* ray, and the less refracted or *extraordinary* ray. The less refracted or extraordinary ray does not obey the ordinary laws of refraction, but presents an image which moves when the crystal is rotated. Both rays are said to be polarized—*i.e.*, consist now of vibrations in one direction only in the plane perpendicular to the line of propagation of the ray.

If a crystal of spar be cut through its obtuse angles, the sections polished and cemented together, and the long sides blackened, a Nicol prism is formed. Such a prism absorbs the ordinary ray by the black sides after it has been totally reflected by the cut surfaces, whilst it allows the extraordinary ray to pass through in a direction parallel to the source of light.

A polarimeter consists of two Nicols mounted parallel to each other—one, the polarizer, fixed; the second, the analyzer, movable. If the movable prism is exactly parallel to the fixed, a beam of light will pass through it; if not exactly parallel, but inclined at an angle, less light will pass through; if at right angles, all light will be cut off.

If a solution of an optically active substance be interposed between the Nicols set parallel, the quantity of light passing through is diminished, but the original intensity can be recovered by rotating the analyzing prism. The amount of such rotation is equal to the power of rotation of the solution. In all polarimeters the analyzer is mounted on a graduated circle, so that the number of degrees of rotation can be easily measured.

The recognition of equal intensity of light in a polarimeter before and after the passage of light through an optically active solution is very difficult, and readings are accordingly far from correct. To overcome this difficulty, Laurent placed behind the polarimeter a quartz plate of such *thickness* that one of the two light rays produced in it is retarded by half a wave length (and consequently reversed in direction), and of such *size* that it covered half the field.

The ray resulting from the blending of the affected and unaffected rays accordingly emerges in a plane at an angle to the original plane; in other words, the polarized light passing through the quartz plate is rotated through an angle. Two sets of rays of polarized light at an angle to each other will accordingly reach the analyzer. If the analyzer be arranged parallel to the light coming from the covered portion, this half of the field will appear light, and the other half dark. On the contrary, if the analyzer be set parallel to the light coming from the uncovered portion, this half of the field will appear light, and the other half dark. By adjustment the analyzer can be placed in a position in which the two halves appear equally illuminated. This position corresponds with the zero of the circular scale and with the zero of the vernier. With Laurent's polarimeter monochromatic sodium light is used, a cell containing potassium bichromate being placed in front of the polarizer to intercept the blue rays.

The instrument consists of the following parts: Bichromate cell, polarizing prism, quartz plate covering half field, trough to carry solution under examination, circle graduated in degrees, a double vernier attached to analyzing prism and moving on graduated circle, and a telescope to focus edge of quartz plate.

When the active substance in solution is placed between the Nicols and equal illumination of the two half fields restored, the vernier will have moved in the direction of the hands of a clock (dextro-rotatory), or in the opposite direction (lævo-rotatory). The distance traversed measured on the circular scale gives the amount of rotation in degrees.

The specific rotatory power $[\alpha]_D$ —that is, the rotation produced by 1 gramme of the substance dissolved in 1 c.c. of liquid examined in a tube 1 decimetre long (rotatory power of 100 per cent. solution)—being known, we can determine the strength of an unknown solution.

For glucose $[\alpha]_D = +52.5^\circ$; lactose $+52.4^\circ$; sucrose $+66^\circ$; galactose $+82^\circ$; maltose $+137.7^\circ$; fructose -93.8° at 20°C .

The percentage strength of a solution is given by the formula—

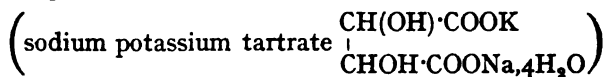
$$[\alpha]_D = \pm \frac{a \times 100}{c \times l}.$$

where $[\alpha]_D$ = specific rotation for sodium, or D light.
 α = observed rotation on the circular scale.
 c = concentration.
 l = length of tube in decimetres.

Polarimetric Estimation of Lactose in Milk.—Put 60 c.c. milk in a 100 c.c. flask; add 1 c.c. mercuric nitrate (Hg dissolved in twice its weight of HNO_3 , specific gravity, 1.42 + an equal volume of H_2O), and fill up to the mark with water. Shake thoroughly and filter through a moist filter-paper. When quite clear, determine rotation in polarimeter. Make several readings, and take an average. Correct for space occupied by proteins and fat. The volume of the fat in c.c. is found by estimating its weight and multiplying by 1.075, and the volume of the proteins is found by multiplying their weight by 0.8. Water equal to the sum of these volumes in c.c. is added to the 100 c.c.

The calculation involved by taking 60 c.c. of milk may be avoided by taking a simple multiple of the standard amount of the polarimeter used. Thus, in the case of an instrument adjusted so that 20.56 grammes lactose in 100 c.c. of solution produce 100 degrees on the percentage scale, 61.68 grammes (20.56×3) are weighed, treated with mercuric nitrate solution, and made up to 100 c.c. The volumes of fat and proteins are calculated, and the sum added to the 100 c.c. Finally the polarimeter reading divided by 3 will give the percentage of hydrated lactose.

Estimation by Fehling's Method.—This method depends on the fact that, whilst Fehling's solution may be boiled without change if a small quantity of glucose or other reducing sugar be added at the boiling temperature, a precipitate of cuprous oxide is formed, and that the amount of copper salt reduced is proportional to the quantity of sugar used. Prepare Fehling's solution. Powder and press between blotting-paper to remove moisture crystals of pure copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$); weigh 69.28 grammes; dissolve in water; add 0.5 to 1 c.c. pure H_2SO_4 ; dilute with pure water to a litre. Weigh 350 grammes Rochelle salt



and dissolve in about 700 c.c. water; weigh 100 grammes NaOH

prepared with alcohol; dissolve in about 200 c.c. pure water, mix the solutions, and make up to a litre.

These two solutions are kept separate, and mixed in equal proportions immediately before use. Each c.c. of the mixture should contain 0.03464 gramme cupric sulphate, which corresponds with 0.005 gramme anhydrous grape sugar and 0.006786 gramme lactose.

For estimation of lactose, Pavy's modified Fehling process is preferable. Pavy added ammonia to the ordinary Fehling solution to prevent the precipitation of cuprous oxide. The end reaction is fixed by the disappearance of the blue colour in a perfectly clear solution.

Mix 120 c.c. ordinary Fehling (not 100 c.c., because in ammoniacal solution only 5 molecules CuO are reduced by one molecule glucose, instead of 6 CuO) with 300 c.c. ammonia (specific gravity, 0.88); add 100 c.c. 10 per cent. NaOH or 14 per cent. KOH, and make up to a litre. [1 c.c. = 0.0005 gramme glucose and 0.0006786 gramme lactose.] A little more time should be allowed during titration for the reduction, as Pavy's solution acts more slowly than the ordinary Fehling, but the operation should be completed within three or four minutes, otherwise the ammonia disappears and Cu_2O is deposited.

Weigh 25 grammes milk into a 250 c.c. flask; add $\frac{1}{2}$ c.c. of a 30 per cent. solution of acetic acid; shake well, and stand aside for a few minutes. Dilute with about 100 c.c. boiling water, and add 25 c.c. alumina cream; shake and set aside again. Pour the more or less clear liquid through a wet filter, and finally wash out on the filter the entire contents of the flask. Collect the filtrate, which must be perfectly clear, and make up with water to 250 c.c.

Transfer a portion of the clear sugar solution to a burette. Raise to the boiling-point in a porcelain dish 10 c.c. of the Pavy-Fehling solution. Run in the sugar solution (drop by drop if possible towards the end) until the last trace of blue disappears. The number of c.c. delivered from the burette = 0.006786 gramme lactose. From this calculate the percentage.

If the ordinary Fehling method be used, a difficulty is encountered in determining the end-point—*i.e.*, the instant at which the blue colour is completely discharged. Dilution of the sugar solution with very weak NaOH causes the Cu_2O to separate out. If soda

be used in very large excess, the oxide is kept in solution, as in Pavy's modification. The end-point difficulty is perhaps best met by using Ling's indicator: Dissolve in 10 c.c. water at 45° 1.5 grammes ammonium thiocyanate and 1 gramme ferrous ammonium sulphate; cool immediately; add 5 c.c. strong HCl when a brownish-red solution is obtained. The colour is got rid of by adding a small quantity of zinc dust. To determine the end-point, remove a drop of the reduced copper solution, and mix it with a drop of the indicator on a white surface; when a red coloration ceases to appear, the reduction is complete.

Genuine commercial milk-sugar crystallized from water gives:

Not more than 0.05 per cent. ash.

Solubility at 15° C. = 7.0 grammes per 100 c.c. (with an increase of 0.1 gramme per 100 c.c. for each degree of increase of temperature).

Fall of temperature, 0.5° C.

Birotation ratio, 1.6.

Amount of milk-sugar, 99.5 to 99.9 per cent.

Milk-sugars are adulterated with cane-sugar, maltose, dextrose, and various mineral matters. Cane-sugar can be detected by treating a solution with yeast at 55° C. for six hours. Milk-sugar is unchanged in specific rotatory power, whilst the addition of as little as 1 per cent. cane-sugar produces a marked change. Maltose is detected by a decrease in the birotation ratio; dextrose by an increase in this ratio and a decrease in the fall of temperature.

Adulteration of Milk.—The principal adulterations are the addition of water and the abstraction of cream.

The estimation of the water added is made from the solids not fat, as these solids, in different samples, do not depart so far from the mean as the fat. The legal limit is 8.5 per cent.

Example.—A milk yields 3 per cent. of fat by one of the foregoing processes of estimation, and 11 per cent. of total solids. The solids not fat amount to $11 - 3 = 8$ per cent.

On the assumption, therefore, that 8.5 per cent. represents 100 per cent. pure milk, 8 per cent. will represent 94.1 per cent. pure milk.

$$(8.5 : 8 :: 100 : 94.1).$$

In other words, 5.9 per cent. of water has been added to this sample.

It may be urged that, since a few animals produce milk containing less than 3 per cent. of fat and less than 8.5 per cent. of solids not fat, it is unfair to the dairyman to enforce these figures as legal limits. But the number of animals in a herd producing milk below these standards is so small in proportion to the whole number, that the mixed milks should in all cases not only reach but surpass the standards.

Cane-sugar, starch, dextrin, and other bodies have been added to mask the addition of water by raising the solids not fat; these may be detected by the sweet taste, deficiency in total nitrogen, and by the ash. Starch is denoted by the blue colour formed with iodine. Common salt has been added, and is detected in the ash by increase of Cl. Chalk, carbonate and bicarbonate of soda, borax, fluorides, etc., may be found. Of these, borax and boracic acid are used as preservatives. The employment of preservatives—bodies such as boric acid, formalin, etc.—that prevent the growth of micro-organisms has been much discussed. Some hold that it is better to add these bodies than to allow the milk to decompose; whilst others advocate the exclusion of all such reagents.

There does not appear to be any experimental evidence to show that small quantities of preservatives like boric acid and its compounds exert an injurious effect upon healthy adults, or even upon healthy children; but in the case of weakly infants there is a strong feeling in favour of excluding all such bodies from their food. Salicylic acid holds a somewhat different position. In quantities necessary to prevent the growth of micro-organisms this drug is likely, in certain cases, to produce injurious effects. Besides, it inhibits the action of the digestive enzymes of the alimentary tract. Its use as a preservative has been rightly forbidden in France. Formalin, formal, or formol is a 40 per cent. solution of formaldehyde in water, and in the strength of 1 per cent. has been much used as a milk preservative. It produces a very decided change in casein, rendering it insoluble in the digestive juices. A patented process exists in Germany for converting casein, by the action of formalin, into a substance resembling celluloid. This preservative should be rigorously excluded from all foodstuffs. The question of the addition of preservatives to milk has another aspect. All purchasers of milk expect to get a thoroughly fresh article. Any

procedure allowed the dairyman by which he can retain milk for a number of days puts a premium on the sale of a stale substance. Milk should be consumed on the day on which it is drawn from the animal. Its constitution is such that, apart from sterilization, which can only be legitimately performed by heat, it rapidly decomposes and becomes unfit for consumption. If dairymen were compelled to keep their churns scrupulously clean, and the temperature of the milk during transit sufficiently low, there would be no need for preservatives, and we should hear little of decomposed milk.

It is stated that a mixture of boric acid and borax is more efficacious in preserving milk than either alone, and that 35 grains of this mixture are required to preserve a gallon of milk.

Detection of Boric Acid or Borax—*The Turmeric Test*.—Evaporate 100 c.c. of the milk, which has been made alkaline with caustic soda (0.5 gramme), to dryness; incinerate. Take up a portion of the ash in water, and the remainder in weak HCl. Add to each portion a few drops of freshly prepared turmeric solution, and evaporate to dryness. When boric acid or borax is present, the residue assumes a brownish-pink colour, which changes to dark green on the addition of a solution of sodium bicarbonate. If the watery extract gives no reaction, whilst the acid extract reacts strongly, it may be concluded that borax is present; if the two reactions are of equal intensity, boric acid has been added; and if the reaction produced by the acid extract is stronger than that produced by the watery extract, it is probable that a mixture of the two is present.

If the ash be moistened with dilute H_2SO_4 , methylated spirit added, and the mixture thoroughly stirred and set on fire, a green border will appear on the flame when boric acid is present.

Estimation of Boric Acid.—The following method is recommended by R. T. Thompson: To 100 c.c. milk add 2 grammes caustic soda and evaporate to dryness in a platinum dish. Char the residue thoroughly, and heat with 20 c.c. water. Add HCl drop by drop till all but carbon is dissolved. Transfer to a 100 c.c. flask, and add 0.5 gramme dry $CaCl_2$. Run in a few drops of phenolphthalein, and then a 10 per cent. solution of caustic soda till a permanent pink colour is perceptible, and finally 25 c.c. lime-water. The phosphoric acid is all precipitated as calcium phos-

phate. Make up to 100 c.c., mix, and filter through a dry filter. To 50 c.c. of the filtrate (representing 50 c.c. of the milk) add normal sulphuric acid till the pink colour is gone, then a few drops of methyl orange, and continue the addition of acid until the yellow is just changed to pink. Next add $\frac{N}{8}$ NaOH till the liquid assumes a yellow tinge, avoiding excess of soda. All acids likely to be present at this stage exist as salts neutral to phenolphthalein, except boric acid, which is neutral to methyl orange, and a little carbonic acid, which latter is expelled by a few minutes' boiling. Cool the solution, add a little more phenolphthalein, and as much glycerine as will form 30 per cent. of the solution, and titrate with $\frac{N}{8}$ NaOH till a permanent pink is produced. Each c.c. of $\frac{N}{8}$ NaOH is equal to 0.0124 gramme crystallized boric acid (=0.007 gramme boric anhydride).

Phosphoric acid can be separated from boric acid by precipitation as calcium phosphate, if not more than 0.2 per cent. of crystallized boric acid be present.

It is necessary not to carry the charring further than that required to produce a colourless solution, as excessive heating drives off boric acid.

Richmond and Miller have published a process for estimating boric acid without ashing the milk or removing phosphoric acid: Weigh about 10 c.c. of the milk; add half the bulk of a half per cent. phenolphthalein solution; run in normal NaOH till pink colour appears; boil and titrate back with normal HCl till white, and finally with $\frac{N}{10}$ NaOH, till faintly pink (colour, though faint, is distinct); add 30 per cent. of glycerol and continue the titration with $\frac{N}{10}$ NaOH. [A glycerol blank is done and subtracted if necessary].

The number of c.c. $\frac{N}{10}$ NaOH used for the final titration, multiplied by 0.0062, gives the quantity of boric acid contained in the quantity of milk operated upon.

Formalin.—Pure H_2SO_4 and pure formaldehyde give no colour reaction with proteins. Addition of small quantities of oxidizing substances such as hydrogen peroxide, ferric chloride, sodium peroxide, potassium persulphate, etc., produces a characteristic colour. The reaction fails if the quantity of formaldehyde is increased beyond a certain limit which is in proportion to the amount of oxidizing reagent used. Rosenheim has shown that the formaldehyde

is oxidized, producing an intermediate oxidation product, which then reacts with the protein. He found that the ammonium compound of diformaldehyde-peroxide-hydrate, $\text{OH} \cdot \text{CH}_2\text{O} \cdot \text{O} \cdot \text{CH}_2\text{O} \cdot \text{OH}$, an oxidation product intermediate between formaldehyde and formic acid, reacts with proteins and pure sulphuric acid, producing the characteristic colour. This is a general reaction for proteins, and depends on the presence of tryptophane (indol-amino-propionic acid). The intensity of the reaction with different proteins varies directly as the amount of tryptophane present in the protein molecule, and bodies destitute of tryptophane fail to give the reaction.

Hehner's Test.—To 10 c.c. of milk in a test-tube add 1 drop ferric chloride solution, and dilute the milk to about 30 c.c. To a portion of this in another test-tube add concentrated H_2SO_4 , by cautiously pouring it down the side of the tube so as to form a layer at the bottom of the milk. A violet-blue ring will be formed at the junction of the liquids.

A few c.c. of the milk are curdled by dilute sulphuric acid, and a little Schiff's reagent (a solution of fuchsin decolourized by sulphurous acid) added to the filtrate in a test-tube, which is corked and allowed to stand. In a short time a violet-pink colour is produced in the presence of the aldehyde.

A further qualitative test: Boil 10 c.c. of milk; add a few drops 25 per cent. H_2SO_4 ; filter; to filtrate add 5 c.c. 0.1 per cent. solution of phloroglucin and 5 c.c. 5 per cent. NaOH. A rose-pink colour indicates formalin.

Estimation of Formalin.—There is no satisfactory method of estimating formalin. An approximate estimation, which must be made early, as the aldehyde rapidly disappears, may be carried out by the following method:—Reagents required: a normal solution of H_2SO_4 , a few 100 c.c. bottles, with close-fitting rubber stoppers, and a boiler, in which they may be immersed to the neck, a solution of methyl orange, and an approximately normal solution of ammonia. Place in each bottle 25 c.c. of the ammonia solution, and to half of them add a sample containing 0.5 gramme formaldehyde. Stopper tightly, place the bottles in the boiler, fill with water to the neck, and boil for one hour. Cool slowly, and titrate with the sulphuric acid, using methyl orange as indicator. The differences in the readings of the blanks and the samples represent

the ammonia consumed in normal c.c. Each c.c. = 0.0601 gramme formaldehyde. Any acid that may be present must be accounted for.

The following method originally described by Shrewsbury and Knapp is perhaps as satisfactory as any other: An oxidizing reagent is prepared by adding 0.05 to 0.1 c.c. pure HNO_3 to 100 c.c. concentrated HCl . Add to 5 c.c. of milk in a test-tube 10 c.c. of the reagent; shake vigorously, and place in a water-bath at a temperature of 50°C . In about ten minutes the contents of the tube are cooled to room temperature. A violet colour indicates formaldehyde, and its intensity indicates the amount.

The quantitative estimation is effected by setting half a dozen milk tubes containing known quantities of formalin at the same time as the sample, and at the end of the time allowed for the test selecting the match.

Salicylic Acid.—Precipitate the proteins from 50 to 100 c.c. of milk by the addition of mercuric nitrate, and filter. Shake up the filtrate with half its volume of a mixture of equal parts ether and petroleum ether, and stand aside until the ether separates out. Pipette off the ether, and evaporate to dryness in a clean flask. Dissolve the residue in a few drops of hot water, and add to a portion of the solution a drop of a 1 per cent. ferric chloride solution; in the presence of salicylic acid a violet or purple colour is produced. Add to a second portion of the solution a little bromine water: salicylic acid produces a curdy, yellowish precipitate. Evaporate the third portion of the solution to dryness with strong HNO_3 , and take up the residue in a few drops of water. If salicylic acid be present, a yellow coloration is produced on adding ammonia.

It should be borne in mind that carbolic acid and other hydroxy-benzene derivatives act in a somewhat similar manner to salicylic acid. The colour reaction with ferric chloride remains in the presence of alcohol in the case of salicylic acid, but disappears on addition of alcohol in the case of carbolic acid.

A further test consists in evaporating a part of the ethereal extract to dryness, placing a minute portion of the residue in the subliming cell, and comparing the crystalline sublimate with one obtained from pure salicylic acid. The melting-point of pure salicylic acid is 155° to 156°C .

A quantitative estimation may be approximately made by matching the colour produced by ferric chloride in a standard solution containing 0.05 per cent. salicylic acid in 50 per cent. alcohol. A 1 per cent. iron alum is recommended instead of ferric chloride. Definite amounts of the salicylic acid solution should be added to a milk filtrate resembling as nearly as possible that of the sample.

Benzoic Acid is but very occasionally found in milk. It is detected as follows: Render alkaline with baryta-water 200 c.c. milk, and evaporate down to one-fourth. Mix the residue with CaSO_4 to form a paste, and dry on the water-bath. Powder, moisten with dilute H_2SO_4 , and extract with cold 50 per cent. alcohol. Neutralize the alcoholic extract with baryta-water, evaporate to small volume, acidulate with dilute H_2SO_4 , and extract with ether. On evaporating the ether, any benzoic acid will be found sufficiently pure for testing. Make a watery solution of the benzoic acid, and add a little sodium acetate; now add a drop or two of ferric chloride to obtain a reddish-yellow colour.

Hydrogen Peroxide.— H_2O_2 in the presence of organic matter rapidly splits into water and oxygen. If milk to which it has been added be examined before it disappears, it may be detected by addition of paraphenylene-diamine, when a blue colour is produced. The reaction depends on the presence of an oxidase, which is destroyed by heat; hence if the sample has been heated, it will be necessary to add a little fresh milk. Milk free from H_2O_2 decolourizes Schardinger's reagent (5 c.c. alcoholic methylene blue, 5 c.c. formaldehyde, 190 c.c. H_2O), but milk that has been treated with H_2O_2 ('Buddeized') fails to decolourize the reagent, and only regains this power after bacterial fermentation has taken place.

Sodium Carbonate.—Ash a weighed portion of the milk. The ash of 5 grammes normal milk does not contain more alkalinity than that neutralized by three-tenths of a c.c. of $\frac{N}{10}$ HCl . Excess of alkalinity over this may be regarded as sodium carbonate.

Mix 10 c.c. of milk with 10 c.c. of rectified spirit in a test-tube; add 2 or 3 drops rosolic acid solution (rosolic acid, 1 gramme; alcohol, 25 c.c.; water to a litre). A rose-pink colour indicates sodium carbonate.

A preservative named 'mystin' (a mixture of formaldehyde and

sodium nitrite) has been found in milk. This mixture may be detected by destroying the nitrite in 10 c.c. of milk, with a few c.c. of a 2 per cent. solution of urea, and then testing for formaldehyde; or by distilling off the formaldehyde and applying Griess's test for nitrites.

Colouring Matters.—The most commonly occurring colouring matter is the vegetable substance annatto; carrot juice, turmeric, and saffron are also used. These colouring matters are all soluble in alcohol, but not soluble in water.

To detect annatto, add to a few c.c. of milk a little bicarbonate of soda, and immerse a strip of white filter-paper over night; a brown stain in the paper indicates this substance.

If an alcoholic solution of annatto, saffron, and turmeric be evaporated down to dryness, and a drop of concentrated H_2SO_4 placed on the residue, a dark blue colour is produced, changing to green in presence of annatto and saffron. In the case of saffron a final reddish-brown is formed. Turmeric produces a violet-red turning brown on the addition of an alkali.

Whilst vegetable colouring matters may be regarded as incapable of damaging the digestive organs of man, it is not clear that certain coal-tar dyes are equally innocent. A dilute mineral acid added to milk containing an azo-coal-tar dye gives a pink colour.

Sour Milk.—During the lactic fermentation of milk wherein half the lactose may be transformed into lactic acid and volatile bodies, little or no change takes place in the fats. But before estimating these some preliminary treatment of the curdled sample is necessary. A uniform emulsion is made by means of a whisk. The total solids are estimated on a portion of this in the usual way.

Fat and Non-Fat Solids.—Weigh 10 grammes into a flat tared platinum basin, carrying a glass rod; add 2 drops (0.5 per cent.) phenolphthalein; run in decinormal strontia until alkaline, noting the number of c.c. used; evaporate on a water-bath till the consistency of dry cheese is reached. Pour 20 c.c. of dry ether over the solids and thoroughly triturate with the glass rod. Decant through a dry weighed filter-paper into a weighing flask. Repeat the ether treatment several times. Distil off ether; dry and weigh residual fat.

Transfer the solids completely to a weighing flask; add the filter-

paper which was previously thoroughly washed with ether; dry for three hours at 100° , and weigh; dry for a further two hours and again weigh; dry for another hour and weigh (last two weights should not differ by more than a milligramme). Deduct 0.00428 gramme for each c.c. of strontia used, also the weight of the filter-paper. Result = non-fat solids.

Correction for Alcohol formed from Lactose.—Distil 100 grammes of the milk and neutralize the distillate with $\frac{N}{10}$ NaOH (litmus indicator). Redistil the neutralized distillate and calculate the percentage amount of alcohol from an alcohol table. The percentage weight of alcohol, $\times \frac{90}{100}$ = percentage of lactose that has disappeared in formation of alcohol.

Correction for Volatile Acids.—Determine the total acidity in 10 grammes of the milk by $\frac{N}{10}$ NaOH (phenolphthalein indicator). Weigh another 10 grammes of the sample in a platinum dish, and add half the quantity of $\frac{N}{10}$ NaOH necessary to neutralize. Evaporate to dryness on a water-bath with frequent stirring; add 20 c.c. boiling water and thoroughly detach solids from dish; now add $\frac{N}{10}$ NaOH till neutral. Difference between original acidity and acidity of evaporated portion = volatile acidity recorded as acetic acid; and 60 parts acetic acid (CH_3COOH) = 62 parts original lactose ($\text{CO}_2 + \text{H}_2\text{O}$). Richmond rightly points out that this correction is inaccurate— CO_2 driven off is calculated as acetic acid; all volatile acids are not driven off; there is a possibility of lactic acid being volatile, and it may be converted into a lactose.

Thorpe makes an ammonia correction: 2 grammes of milk are made up to 100 c.c. with ammonia-free distilled water, and filtered through a carefully washed filter. Ten c.c. of the clear filtrate are similarly made up to 50 c.c. in a Nessler glass, and the ammonia estimated by standard ammonium chloride (1 c.c. = 0.01 milligramme NH_3) after Nessler's method.

Richmond shows good reasons for regarding the ammonia correction as unnecessary.

The total correction (0.2 to 0.3 per cent. additive) is fairly constant in properly sealed samples three to six weeks old.

Bacteria in Milk.—Micro-organisms enter milk from the udder, during milking, and during transit and distribution. It is not possible to estimate the total number of bacteria in milk. Perhaps

the best count is that which demonstrates the presence of pollution by manure: *B. coli*, *B. enteritidis sporogenes*.

The methods employed in this work differ in no main principle, from those used in connection with water. The utmost care is necessary in the collection of samples. Dilutions are conveniently made in sterile flasks or bottles by placing 10 c.c. of milk or of a particular dilution in the vessel which already contains 90 c.c. of sterile distilled water.

Estimation of B. Coli.—To a series (better to a double series) of lactose, bile-salt broth tubes are added respectively—1·0, 0·1, 0·01, 0·001, 0·0001, 0·00001, 0·000001 c.c. (and smaller fractions, if necessary, depending on the degree of pollution of the sample.) Record is made of the smallest quantity producing acid and gas in two days at 37° C.

B. Enteritidis Sporogenes.—Add 1, 1·5, and 2 c.c. of the sample to tubes containing 10 c.c. fresh milk recently sterilized. Add 5, 10, and 20 c.c. to empty sterile tubes. Heat the six tubes for ten minutes at 80° C. Cool promptly and incubate anaerobically for two days at 37° C. Look for the characteristic enteritidis changes.

Pathogenic Micro-Organisms — B. Tuberculosis.—Centrifugalize 50 or 100 c.c. of the milk. Examine a portion of the sediment microscopically. Make and fix films on microscopic slides. When thoroughly fixed, wash out all fat with a mixture of equal parts of anhydrous ether and absolute alcohol. Stain by the Ziehl-Neelsen method. Use the remainder of the sediment for inoculating several guinea-pigs subcutaneously on the inner side of the left leg. Evidence of infection may be found at various subsequent dates in enlargement of the popliteal, inguinal, sublumbar, and retro-hepatic lymph glands on left side, and in tubercles in the spleen. Four weeks is an average time for the production of these appearances. When the milk contains large number of *B. tuberculosis*, they may be found as early as fifteen days after inoculation; when few bacilli exist, five to six weeks may be required to give results. It is well to make smears from the enlarged glands and stain with Ziehl-Neelsen's fluid. Inasmuch as certain non-pathogenic acid-fast bacteria presenting morphological characters somewhat similar to *B. tuberculosis*—such as Möller's Timothy-

grass bacilli, Rabinowitch's butter bacillus, the smegma bacillus, *mist bazillus*, and Johne's bacillus—produce tubercular lesions in the guinea-pig somewhat resembling those produced by *B. tuberculosis*, it is not always safe to rely on inoculation. The diagnosis can be established definitely by sowing on glycerin, agar, or other media portions of the pulp of the lymph glands, from which the smears above mentioned are made. All the non-pathogenic organisms will form definite growths in two or three days, whereas *B. tuberculosis* will ordinarily require three or four weeks for growth. It is necessary to investigate the cream in all these details, as well as the sediment.

The Klebs-Löffler Bacillus.—Sediment and cream are investigated morphologically and culturally. If organisms resembling the diphtheria bacillus morphologically are found, they must be grown on blood serum, and their virulence must be tested by animal inoculation.

B. Typhosus.—This organism is almost as difficult to detect in milk as in water. Portions of sediment and cream are applied to those media intended for the differentiation of *B. typhosus*, such as lactose bile salt neutral red agar, followed by subculture on Conradi and Drigalski's medium, malachite green agar, etc.

Streptococci.—In the milk of cows suffering from mastitis, enormous numbers of streptococci are found, and when these are inoculated into the teats of goats, they set up an inflammatory reaction. But since they are found in certain numbers in the milk of healthy cows collected in the most cleanly manner, it is difficult, if not impossible, to estimate their significance. All that can be said at the moment is, that where streptococci exist in milk in large numbers, the indication is to examine the animal for mastitis, ulceration of teats, etc. Streptococci arise from the teats and milk ducts of the udder, in large quantities from manure, in smaller quantities from the air, and may be contributed by filthy vessels, foul water, etc.; and in those cases where they occur in very large numbers, if no inflammatory condition of the udder be found, it may be assumed that their presence is most likely due to manure. They can be readily demonstrated in the sediment by making smears and staining with methylene blue.

Quantitative estimation is effected by inoculating glucose neutral

red broth with 1:0, 0:1, 0:01, 0:001 c.c., etc., and incubating for two days at 37° C. Hanging-drop and stained preparations exhibit definite chains of cocci.

Cellular Elements of Milk.—These are recovered and studied in the sediment microscopically. Where pus enters milk in large quantities, the fact is at once revealed by a microscopic examination. But whether a few round cells resembling dead leucocytes are to be regarded as evidence of a small quantity of added pus or as normal constituents of certain milk remains an open question.

Bacteriological examination of condensed milk, dried milk, and cheese is carried out in the same manner after a thorough emulsion has been made of a definite weight of the substance in sterile water.

Koumis.—The original koumis was made by the Tartars from mares' milk, which is rich in lactose, and readily fermented. This stimulating beverage is now largely made from cow's milk, to which sugar and yeast have been added. It undergoes a multiple fermentation—alcoholic, lactic, and proteolytic.

Kephir.—This is a fermented milk similar to koumis. The proteolytic fermentation is less pronounced, and the alcoholic and lactic fermentations are established by a fungus—kephir grains.

Condensed Milks are found in four forms: (1) Condensed whole milk sweetened; (2) condensed whole milk unsweetened; (3) condensed separated milk sweetened; (4) condensed separated milk unsweetened. The process of condensing unsweetened milk appears to kill all bacteria, and organisms that are found in this variety, according to Gordon, are introduced subsequently from the air. In all the sweetened varieties streptococci, with characters similar to those found in milk, were discovered by the same observer.

The chemical and bacteriological examinations of these modifications of milk are worked out on the same lines, as in the case of ordinary milk, condensed milks being first mixed with a definite measured quantity of distilled water.

Dried or powdered milk is produced (1) by applying the fluid in a thin stream to the surface of a heated revolving metallic cylinder, or (2) by passing it in the form of a fine spray into a hot-air chamber. It should contain fully 27 per cent. of fat, and about 32 per cent. each of proteins and sugar.

The fat is best estimated by Adam's process, and the proteins by Kjeldahl's total organic nitrogen process, using the factor 6.38.

Cream is prepared by centrifugalizing milk, and contains 45 to 65 per cent. of fat. Cream is artificially thickened with gelatin, starch paste, condensed milk, and saccharate of lime. Gelatin may be detected by drying a weighed quantity and washing out the fat with ether. The residue, when dissolved in boiling water, will contain the gelatin, which sets on cooling. Or mix a weighed quantity of cream with warm water; precipitate proteins and fat with acetic acid; filter; add to the clear filtrate a little strong solution of tannin, when, if gelatin be present, a voluminous precipitate falls out. A control sample of genuine cream should be operated on in the same way. It will give but a slight precipitate.

Starch is discovered by the blue colour it forms with a solution of iodine.

Calcium saccharate is determined as CaO in the ash. The dicalcium phosphate, tricalcium phosphate, calcium citrate, and lime united with proteins in normal cream, when transformed into CaO, amount to about 22.5 per cent. of the ash.

Cane-sugar in cream is detected by the rich red colour produced when to 15 c.c. of cream, 0.1 gramme of resorcinol, and 1 c.c. of concentrated HCl are added, and the mixture raised to the boiling-point.

BUTTER.

Butter is produced from milk or cream by churning. The agitation causes the fat globules to coalesce to form granules of a fine spongy nature. When butter is collected and worked, it assumes a more homogeneous appearance.

The mean composition of butter made from ripened cream, according to Storch, is:

| | | | | | Per Cent. |
|------------|----|----|----|----|-----------|
| Fat | .. | .. | .. | .. | 82.97 |
| Water | .. | .. | .. | .. | 13.78 |
| Proteins | .. | .. | .. | .. | 0.84 |
| Milk-sugar | .. | .. | .. | .. | 0.39 |
| Ash | .. | .. | .. | .. | 0.16 |
| Salt | .. | .. | .. | .. | 1.86 |

The composition of different butters varies considerably.

If butter be churned at a higher temperature than 13° to 18° C.,

it will contain more water than at medium temperatures. Very low temperatures and rapid churning produce an article containing too much water.

Butter is adulterated with various foreign fats, animal and vegetable, under the name of margarine, which as a rule are little inferior in nutritive qualities to the fat of milk. In the production of margarine, animal and vegetable fats are melted, filtered through coarse filters, and worked up with milk, to look and smell like pure butter.

It is stated that margarine, as prepared for the market, is not quite so digestible as butter. Whether or not this be true, it is illegal to substitute margarine for butter, and the principal object of a butter analysis is to determine the presence or absence of foreign fats.

The odour and taste of butter are characteristic, and excellent tests of its purity. By heating it to 25° C. any unpleasant taste that it may possess becomes more apparent.

Adulteration.—Foreign fats are the chief item of adulteration. Colouring matters, especially annatto, are employed. Water is worked into butter for the purpose of increasing its weight; but, as the addition of water renders butter liable to decomposition, it is only possible to escape detection in cases where the butter is rapidly disposed of. The addition of pepsin, rennet, etc., to milk before churning aims at increasing the yield of butter by securing an increase of contained water.

The Estimation of Water.—At present butter is allowed to contain 16 per cent. of water. The following two methods readily determine the amount of water; the second is the more accurate.

1. Weigh out 10 grammes of butter into a small platinum or porcelain basin provided with a piece of glass rod. Heat on a sand-bath or over a small flame, and carefully stir until all frothing ceases. It is necessary to regulate the temperature so that the curd is not appreciably browned during the heating, and that there is no loss by spirting. The basin with its contents is cooled in a desiccator and weighed. The loss of weight represents the water in 10 grammes.

2. Fill a small platinum or porcelain basin with pieces of pumice that have been recently washed and ignited. Select portions of butter from three different regions of the sample (water is not always

equally distributed throughout the mass of butter), and place them in a clean, wide-mouthed, stoppered bottle. Melt at as low a temperature as possible, and shake vigorously until the mass is solid. Place 5 grammes of this mass in the porcelain basin, and heat in a drying-oven with good draught at 100° C. for an hour. Cool and weigh. Replace in the oven for a further half-hour, and again cool and weigh. Repeat the heating and weighing until a constant weight is obtained. The difference between the lowest weighing and that of the original butter is taken as water.



Beaker for melting
crude butter.

Bottle beaker with funnel
for filtering butter-fat.

Graduated test-tube
for Valenta test.

Platinum
basin.

FIG. 28.

The following table represents the variations of water in Danish butters:

| Percentage of Water. | | | | Number of Samples. | |
|----------------------|----|----|----|--------------------|-----------------|
| | | | | Summer. | Winter. |
| 9 to 10 | .. | .. | .. | 1 | 1 |
| 10 „ 11 | .. | .. | .. | 16 | 8 |
| 11 „ 12 | .. | .. | .. | 136 | 20 |
| 12 „ 13 | .. | .. | .. | 335 | 138 |
| 13 „ 14 | .. | .. | .. | 534 | 431 |
| 14 „ 15 | .. | .. | .. | 512 | 562 |
| 15 „ 16 | .. | .. | .. | 287 | 447 |
| 16 „ 17 | .. | .. | .. | 124 | 205 |
| 17 „ 18 | .. | .. | .. | 39 | 95 |
| 18 „ 19 | .. | .. | .. | 13 | 20 |
| Above 19 | .. | .. | .. | 4 | 3 |
| Average | .. | .. | .. | 14.03 per cent. | 14.41 per cent. |

Butters containing 13.5 per cent. of water are said to have the best flavour.

Estimation of Curd and Salt.—The residue from the determination of water is taken and melted at a low temperature. Ether is added, and the whole well stirred and set in a warm place until the ether is quite clear, when the fluid is decanted into a small weighed flask. Fresh ether is poured on the residue, and when clear poured off. This treatment, repeated three or four times, removes the whole of the fat. A little practice and ordinary care will prevent any of the non-fatty solids being poured away with the solvent. The residue is dried in the hot-air oven to constant weight, and represents salt and solids not fat.

The Salt.—To estimate the salt, the residue from the last determination is treated with hot water and filtered. The filter with its contents is well washed, and the filtrate, when cold, is titrated with standard nitrate of silver, using a 5 per cent. solution of neutral potassium chromate as indicator. The amount of sodium chloride is easily calculated. The silver nitrate should be standardized on pure sodium chloride.

Curd.—The estimation of the proteins is best effected by Kjeldahl's process for the estimation of total N on the residue left after estimating the fat, and multiplying the N by 6.38.

The Fat.—This item is estimated by subtracting the combined weights of water, salt, and solids not fat from 100. As a control the ethereal extract may be evaporated, and the fat residue weighed.

Preservatives.—Besides salt, several substances are used as preservatives, such as boric acid, borax, formalin, salicylates, sulphites, and nitrates. These are all estimated in the watery fluid which separates out underneath the fat on heating the butter.

The estimation of **boric acid** is carried out as follows: Heat 10 grammes of butter in a dish; wash out the melted mass into a separating funnel with about 50 c.c. boiling water; shake thoroughly, and when fat is separated run off the water into a 100 c.c. flask. Repeat this treatment twice, using less than 25 c.c. boiling water each time. When all the washings are collected in the flask and cold, make up to the 100 c.c. mark. Filter through a dry filter, and titrate 50 c.c. as described on p. 175. The titration may be

performed on this solution without any treatment, as butter is free from phosphates.

Formalin cannot be estimated with any degree of exactitude, as it enters into combination with the proteins, so that the uncombined formalin alone reacts, and this gives no information as to the amount originally added.

To estimate **salicylic acid** treat 20 grammes of butter with a solution of sodium bicarbonate several times in a separating funnel: salicylic acid is converted into sodium salicylate. Acidify the extract with dilute H_2SO_4 , and extract with ether; evaporate the ether, and to the residue add a little mercuric nitrate, forming a precipitate nearly insoluble in water. Filter the precipitate off, and wash it with water. From the washed precipitate liberate free salicylic acid with dilute H_2SO_4 . Redissolve in ether, evaporate, and dry residue at 100°C . Extract the residue with petroleum ether, and add an equal volume of 95 per cent. alcohol. Titrate with $\frac{N}{10}$ KOH (phenolphthalein indicator). [1 c.c. $\frac{N}{10}$ KOH = 0.0138 gramme salicylic acid.] Processes which depend on the precipitation of proteins and detection of preservatives in the filtered liquid are liable to error owing to the great solubility of salicylic acid and benzoic acid in butter-fat. The extraction of the fat with solvents (ether, alcohol, chloroform, etc.) frequently gives rise to troublesome emulsions. To overcome these troubles Monier-Williams has devised a method of detecting small quantities of benzoic acid, saccharin, and salicylic acid in cream: Acidify 100 grammes of cream with 1 c.c. concentrated phosphoric acid; heat with constant stirring either in a porcelain dish on gauze over a Bunsen or on a boiling-water bath *in vacuo* (temperature should not rise above 120°C .) until all water is expelled. At least 95 per cent. of the salicylic and benzoic acids remain in the fat, and only the merest traces escape in the steam. Filter the clear fat through a dry filter. Allow the fat to cool to 60° to 70°C .; shake with 50 c.c. of 0.5 per cent. sodium bicarbonate previously heated to 60° to 70°C .; when separated from the fat filter the alkaline liquid through a wet filter; acidify with 1 c.c. concentrated HCl; cool, and extract three times with 15 to 20 c.c. ether. Dry combined ether extract with CaCl_2 , and distil off ether. The residue will have a distinctly sweet taste if saccharin be present. Stir the residue on

a water-bath with 1 c.c. strong ammonia; evaporate to dryness; add three or four drops of water and a drop of 10 per cent. iron alum solution on a glass rod. The characteristic purple colour appears in presence of salicylic acid, and a buff-coloured precipitate in the presence of benzoic acid. The method is said to detect with certainty in 100 grammes of cream the following quantities of these preservatives occurring singly or all together: 0.0075 per cent. benzoic acid; 0.001 per cent. saccharin; 0.0002 per cent. salicylic acid.

Sulphites.—A portion of the watery liquid is distilled with dilute HCl, and the gas evolved is passed into $\frac{N}{10}$ I solution, which in turn is titrated with sodium thiosulphate. Sixty-four parts of SO_2 are converted into sulphuric acid by 254 parts of I. Or the SO_2 gas may be passed into bromine-water, and the H_2SO_4 formed, estimated as $BaSO_4$. Sixty-four parts SO_2 represent 233.5 parts $BaSO_4$.

Butter-Fat: Preparation of Fat for Analysis.—A portion of the sample of butter is placed in a beaker and heated at a temperature of 45° to 48° C. in an air-oven. In a little time three layers separate out in the beaker. The largest, the butter-fat, containing a few particles of curd in suspension and a few drops of water underneath the surface film on the top; a greyish-white layer, the curd, near the bottom; and underneath a small quantity of water. If the sample be genuine butter, the melted fat is quite transparent, whereas if mixed with margarine, melted, and re-emulsified, churned at a high temperature, or rancid, it is generally turbid.

The fat is poured on a dry filter kept at a temperature above the melting-point of butter, and is now free from the other constituents, except about 0.1 per cent. of water, and a trace of lactic acid. These manipulations are readily carried out by placing the beaker containing the melted butter, and a second beaker (carrying a funnel and filter-paper) in which the fat is received, on the top of an air-oven whose inner temperature approaches but does not exceed 50° C. After filtration the fat is rapidly cooled so as to prevent partial solidification, and to obtain a homogeneous mass.

Butter-fat contains considerable quantities of the glycerides of the fatty-acid series $C_nH_{2n+1}COOH$, of low molecular weight.

The lowest and most important is butyric acid. Acids of the oleic series are also present.

The various foreign fats which are admixed with butter, such as beef and mutton fats, lard, cottonseed oil, and other vegetable oils, present several important physical and chemical differences.

Physical Properties of Fats: 1. Melting-Point.—Fats are not single substances, but mixtures of different glycerides; the melting-points are therefore not sharp. The melted fat is drawn into a capillary tube 1 millimetre bore, so as to give a column about 1 centimetre in length. Not less than a day should elapse before the test, as even pure glycerides of fatty acids that are single chemical entities melt at a much lower temperature if they have been recently melted than that at which they melt if they are kept in the solid state for some time. The capillary is attached by a rubber band to the stem of a delicate thermometer, reading tenths of a degree, so that the column of solidified fat is opposite the thermometer bulb. The thermometer and its attached capillary tube are then immersed in water in a test-tube, and the test-tube in turn is immersed in a beaker of water mounted on gauze over a Bunsen burner. The water in the beaker is heated gradually (rise of temperature not to exceed 0.5° C. per minute), and the exact temperature noted at which fusion of the fat occurs: this is the melting-point. The flame is removed, and the temperature noted at which the fat solidifies: this is the solidifying-point. Butter-fat melts at about 33° C. Some foreign fats have melting-points lying very near to that of butter-fat; moreover, artificial butters are made to melt at the same temperature as butter, so this test is of little practical value in distinguishing pure butter from margarine.

2. Specific Gravity.—On account of the glycerides of low molecular weight which it contains, butter-fat has a greater density than the fats used to adulterate it. As it is more convenient to take the specific gravity of a fluid than a solid, and as Skalweit found that at, or around, the temperature 38° C. there is the greatest difference between the specific gravities of butter and foreign fats, this temperature is usually adopted for the taking of specific gravities.

Fill the pycnometer with water at 38° C. and weigh it. Remove

the water and dry the flask in an air-oven through which a good current of air passes. Now fill it with fat, and place it in water at 38°C . till the volume is constant. Weigh again rapidly, and the weight of the fat divided by the weight of water gives the specific gravity at 38°C . The limits of specific gravity for pure butter-fat at 38°C . are 0.914 and 0.909. The fats usually added as adulterants have a mean specific gravity of 0.903.

The density may also be determined by a hydrometer or by Westphal's balance at 38°C .

The presence of glycerides of lower fatty acids raises the specific gravity of a fat; hence rancidity is accompanied by an increase in the specific gravity.

A pycnometer with capillary side-tube can be used for estimating the specific gravity of solid fats: the bottle is filled with water and weighed; a weighed amount of the solid fat is introduced and the stopper inserted. The diminution of weight plus the weight of the solid fat gives the amount of water displaced and the volume of the fat.

3. Solidification-Point and Titre Test.—When melted fat is cooled, the temperature falls gradually to a variable degree, then rises rapidly to a certain constant temperature, at which it remains steady for a time before it begins to fall again; this maintained temperature is the solidification-point. This test is generally carried out on the fatty acids obtained by saponification of the fats, and is then known as the 'Titre test.' A method of carrying it out is the following: Saponify 75 grammes of the fat in a metal dish with 60 c.c. of 30 per cent. NaOH and 75 c.c. 95 per cent. alcohol, or 120 c.c. water. Evaporate to dryness and dissolve in 1 litre of water. Boil to remove alcohol. Add 100 c.c. 30 per cent. H_2SO_4 , and heat till clear: the fatty acids are separated. Wash them with hot water till free from soluble acids, and filter through a dry filter on a hot-water funnel. Dry for twenty minutes at 100° , and cool down to within 15° to 20° of the solidification-point. It is important that the fatty acids be thoroughly dried. Pour into a test-tube 100 millimetres long and 25 millimetres in diameter, which is suspended by a cork in the mouth of a jar 70 millimetres wide and 150 millimetres high. A thermometer, graduated in tenths of a degree, between 10° and 60° , with a bulb 3 centimetres long by

6 millimetres diameter, is made to act as a stirrer. The determinations should not vary by more than 0.1° C.

4. The Refractive Index : Oleo-Refractometry.—The oleo-refractometer measures the refraction which a ray of light undergoes in passing through a layer of butter. It is found more convenient to read the angle of total reflection, as indicated by the sharp colourless border-line which vertically intersects the scale of the instrument between the light and dark sections of the field of view. A few drops of the butter-fat to be tested are poured warm into the prism of the instrument, and the deviation noted at a temperature of 45° C. Pure butter gives a deviation of about 30° to the left. Certain forms of margarine give deviations much less— 15° and 20° —whilst cocoa-nut oil gives over 55° .

The butyro - refractometer of Zeiss is a modification of the Abbé refractometer, and gives rapid readings in scale divisions which by reference to a table can be read off as refractive indices.

5. Microscopic Examination.—When examined microscopically butter-fat presents a collection of small round refractile globules, together with a few larger globules fairly uniform in size and in the number present in a single field. Margarine presents a mass of small globules much less distinct in outline and more crowded together. The larger globules occur in relatively greater numbers, and present much more diversity in size.

Chemical Methods used in Analysis of Fats : 1. The Acid Value.—This is represented by the number of milligrammes of KOH required to neutralize a gramme of the fat. It is accordingly a measure of the degree of hydrolysis of the fat which may be due to rancidity or to ferment action. Five to ten grammes of the fat are dissolved in alcohol and titrated against tenth normal alkali in presence of phenolphthalein or alkali blue, 6b of Meister, Lucius, and Brüning.

2. The Saponification Value is a measure of the mean molecular weight of the fatty acids entering into the composition of a fat. It is to be noted that in the titration of fatty acids soaps are hydrolysed by water, and accordingly react alkaline; such hydrolysis is prevented if 40 to 50 per cent. of alcohol be present. The saponification value is given by estimating the number of

milligrammes of KOH neutralized in saponification of 1 gramme of the fat by the total fatty acids that it contains, whether originally combined with glycerol or other alcohol, or free. Heat 2 grammes of fat with 25 c.c. of alcoholic potash in a Jena flask (glass that does not give off alkali) under a reflux condenser for half an hour. Carry out a blank control with the same volume of alcoholic KOH in a similar flask lest the titre be altered by CO_2 or other agency during heating. When the saponification is complete titrate the alkali in each flask with $\frac{N}{2}$ HCl and phenolphthalein. The difference between the amounts of acid required by the two flasks gives the amount of alkali neutralized by the fatty acids contained in and liberated during saponification from the amount of fat taken; from this the saponification value is calculated.

3. **The Hehner Value.**—This is the percentage of fatty acids insoluble in water produced on saponification by a fat.

Two or three grammes of the fat are saponified with alcoholic potash. The saponified mass is washed with hot water into a beaker on a steam bath, and acidified with dilute H_2SO_4 . When the subjacent aqueous layer has become clear, the contents are filtered through a weighed filter; it is well to half fill the filter with water before pouring the fatty acids on. The beaker is washed with a jet of hot water, and the acids washed continuously as long as any acid reaction can be detected in the washings. The filter with its funnel are then immersed in cold water, so that the fatty acids solidify; the filter is dried and weighed, or when dry it may be extracted in a Soxhlet apparatus with petroleum ether, the ether evaporated, and the residue dried and weighed.

The Hehner value of butter is between 86 and 88, of triolein 95.7, of lard and most oils about 95.

4. **The Iodine Value.**—This value gives the amount of halogen reckoned as iodine that the unsaturated acids in the fat take up, expressed as a percentage by weight of the fat. Triolein, for example, whose molecular weight is 884, takes up 6 atoms of iodine ($6 \times 127 = 762$), or 86.2 per cent.; oleic acid has an iodine value of 90.1.

As saturated acids and their glycerides absorb no halogen, the iodine value is a measure of the amount of unsaturated acids

present. Acids with unsaturated bonds in more than one place absorb proportionately more iodine.

Determination by the Method of Wijs.—Prepare a titrated solution of iodine monochloride, a titrated solution of sodium thiosulphate, and a 10 per cent. solution of KI.

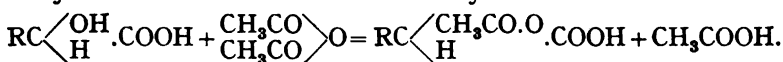
The monochloride is obtained thus: Weigh 9.4 grammes iodine trichloride into a 300 c.c. flask, pour in 200 c.c. glacial acetic acid, fit the flask with a cork through which passes a CaCl_2 tube; heat on a water-bath till the contents are dissolved. Weigh 7.2 grammes of iodine, which has been thoroughly pulverized in a mortar, into a second flask; wash out the mortar with glacial acetic acid, and heat this flask as the other. Pour the contents of the two flasks into a stoppered litre flask. Any undissolved iodine is further heated with additional acetic acid till all is dissolved and added to the litre flask. This flask is then stoppered and allowed to cool; when cold, the solution is made up to a litre with acetic acid and titrated next day. The strength of the iodine chloride solution is likely to alter a little in the first twenty-four hours, but after that remains fairly constant for some weeks if care has been taken to exclude all water from the glacial acetic acid. To do the titration pipette into an Erlenmeyer flask exactly 20 c.c. of the iodine chloride solution; add about 10 c.c. of the KI solution and about 300 c.c. of water. Run in a standard sodium thiosulphate solution (24 grammes to a litre standardized by Volhard's method), and finish off with starch solution. From the amount of thiosulphate used the amount of iodine in the measured amount of Wijs's solution is calculated.

Estimation of the Iodine Value of the Fat or Fatty Acid.—Weigh into a stoppered flask of 100 to 150 c.c. capacity a quantity of the fat or fatty acid depending on the iodine value of the Wijs's solution used (there should be two or three times as much iodine in the Wijs as the fat can absorb), say 0.2 to 0.5 gramme, and dissolve it in 10 c.c. CCl_4 ; add, say, 25 c.c. Wijs, stopper and stand aside for a couple of hours in the dark. Now pour the contents of the flask into an Erlenmeyer (half to litre size); wash out any traces of iodine with 10 c.c. of the KI solution and afterwards with water; the bulk of fluid obtained should be about 300 c.c. Lastly titrate with thiosulphate and calculate the unabsorbed iodine.

This figure, subtracted from the amount of iodine contained in the Wijs used, gives the iodine absorbed, which is readily calculated into a percentage of the amount of fat taken.

5. The Acetyl Value.—This determination gives the number of milligrammes of caustic potash required to neutralize the acetic acid liberated when 1 gramme of acetylated fat or fatty acids is saponified.

Those fatty acids in a fat or oil which are hydroxy become acetylated when heated with acetic anhydride:



The number of acetyl radicals taken up depends on the number of hydroxy acids present, and the number of hydroxyl groups contained. On saponification, the acetyl groups are split off as acetic acid, and the amount of acetic acid so liberated is a measure of the hydroxyl groups.

Heat 5 to 10 grammes of the fat or fatty acids with twice the weight of acetic anhydride in a flask under a reflux condenser for a couple of hours. Transfer the contents to a large beaker, and add $\frac{1}{2}$ litre of boiling water. Heat for half an hour whilst a stream of CO_2 is led through to prevent bumping. Separation is allowed to take place, and the watery layer is syphoned off. Salt may be added if the oily layer does not separate easily. More water is added, and in turn syphoned off till the acetic acid formed from the excess of anhydride has all been removed. Filter through a dry filter in a drying oven to remove water.

Weigh 3 to 5 grammes of the acetylated product; saponify with a known amount of KOH and determine the saponification value. Free the soap from alcohol by evaporation, and estimate the acetic acid as follows: Add an excess of 10 per cent. H_2SO_4 , and distil the liquid in a current of steam. Collect the distillate until 100 c.c. require not more than 0.1 c.c. of decinormal alkali to neutralize it. More than 600 c.c. of distillate will generally be obtained. Titrate this with decinormal alkali (phenolphthalein indicator), and multiply the number of c.c. used by 5.61; divide the product by the weight of the acetylated product used to get the acetyl value.

6. The Reichert-Meissl Value.—This is that usually applied for identification of butter-fat. It is a measure of the amount of lower

fatty acids in a fat which volatilize in a current of steam. The value is expressed by the number of c.c. of $\frac{N}{10}$ alkali required to neutralize the volatile fatty acids liberated under certain prescribed conditions from 5 grammes of the fat. In this country the Wollny modification is used. Most fats and oils in the fresh state contain only traces of volatile acids or their glycerides, and give values less than one. Cocoa-nut oil gives Reichert-Meissl-Wollny value 5 to 8; butter a notable exception possessing a value 26 to 32. Some porpoise oils are said to reach 60.

The estimation is a comparative one, and that only whilst the conditions are accurately observed.

Weigh 5 grammes of *prepared* butter-fat into a flat-bottomed flask of 300 c.c. capacity, having a neck 7 to 8 centimetres long by 2 centimetres wide. Add 2 c.c. NaOH solution prepared by dissolving 98 per cent. NaOH in an equal weight of water (protected from the action of atmospheric CO_2) and 10 c.c. 92 per cent. alcohol. Heat the flask on a boiling bath for fifteen minutes under a reflux condenser. Remove the condenser, and drive off the alcohol completely by heating further for half an hour. Add 100 c.c. of water which has been boiled (to remove CO_2), and heat till the soap dissolves. Add 40 c.c. of normal sulphuric acid and some bits of pumice or porous clay, and connect the flask with a condenser tube 7 millimetres in diameter, surrounded by a water-jacket 35 centimetres long by means of a bent tube 15 centimetres long from the cork of the flask to the bend of the tube, on the middle of which a bulb, 5 centimetres in diameter, is blown. The flask is heated on an asbestos board, with an opening in its centre 5 centimetres in diameter, by a small flame till the insoluble acids are melted. When fusion is complete the heat is increased, and 110 c.c. are distilled in about thirty minutes into a graduated flask. Shake the distillate and filter off 100 c.c. into a beaker; add 0.5 c.c. of a 1 per cent. solution of phenolphthalein in alcohol, and titrate with $\frac{N}{10}$ soda or baryta. Carry out a blank experiment with the same quantities of everything except fat; the amount of $\frac{N}{10}$ alkali required to neutralize the distillate should not exceed 0.2 to 0.3 c.c. The number of c.c. decinormal alkali used, less the blank, multiplied by 1.1, gives the Reichert-Meissl-Wollny number.

Leffmann and Beam employ 20 c.c. of glycerol instead of the

alcohol used by Wollny. They heat the fat with glycerol and soda for eight minutes, when the fluid becomes clear and is allowed to cool to about 80° C. Then 90 c.c. of water at about 80° C. and 50 c.c. of a 2.5 per cent. H_2SO_4 solution are added, and the process is finished as above.

In this distillation only a part of the volatile acids distils over [87 per cent. of total volatile acids (according to Richmond); 88 per cent. butyric, 88 to 100 per cent. caproic, 24 to 25 per cent. caprylic (according to Jensen)].

Five grammes of pure butter-fat give a number never less than 24, margarine never more than 3.

In order to prevent fraud not more than 10 per cent. of butter-fat is permitted in margarines, which will produce a Reichert-Wollny number of 4.

Example.—In a mixture of margarine and butter-fat the Reichert-Wollny figure is 16; find the percentage of butter-fat.

Taking 3 as the highest possible figure for margarines, and 24 as the lowest for butter-fats, 21 (24 - 3) will represent 100 per cent. of pure butter-fat.

$$21 : 16 - 3 :: 100 : x.$$

$$x = \frac{13 \times 100}{21} = 62 \text{ nearly.}$$

This sample, therefore, contains 62 per cent. of butter-fat, and consequently 38 per cent. of margarine.

The Polenske Number.—This number represents the volatile fatty acids insoluble in water. It is much used in detecting coconut oil in butter and other fats. It may be determined with the Reichert-Meissl figure in one weighed portion of the fat.

Saponify 5 grammes of prepared fat with 20 grammes of glycerol and 2 c.c. of a 50 per cent. NaOH solution. This requires about five minutes, and is complete when the liquid is quite clear. While still hot add 90 c.c. of boiled water, at first drop by drop, to prevent frothing, and shake till the soap is dissolved. Warm to 50°, and add 50 c.c. dilute H_2SO_4 (25 c.c. to a litre) and $\frac{1}{2}$ gramme of granulated pumice (grains 1 millimetre in diameter). Connect with distilling apparatus used in the Reichert-Meissl method, and distil over 110 c.c. in twenty minutes. Cool the flask by immersion in water at 15° C. Stopper it, and invert four or five times. Filter

through a dry filter fitted close to the funnel (100 c.c. of the filtrate may be titrated for the Reichert-Meissl number). Wash the material on the filter with three 15 c.c. portions of water, each of which have washed out the flask and the condenser. Dissolve the fats on the filter with three 15 c.c. portions of neutral 90 per cent. alcohol. Titrate the united alcoholic washings with $\frac{N}{10}$ barium hydrate, using phenolphthalein as indicator. The number of c.c. used is the Polenske number.

Samples of butter possessing Reichert-Meissl figures 25 to 30 will give Polenske numbers of 1.5 to 3.

Samples of cocoa-nut oil of Reichert-Meissl values 6 to 7 will give Polenske figures 16 to 17.

Lard and tallow give Reichert and Polenske figures of about 0.5 each.

Valenta's Test.—Valenta demonstrated the fact that there is a considerable difference in the temperatures at which various fats dissolve without turbidity in acetic acid.

Weigh out 2.75 grammes of butter-fat into a test-tube; add 3 c.c. 99.5 per cent. acetic acid; insert a thermometer, and gently heat with vigorous shaking until the mixture becomes transparent. Now cool down gradually, stirring with the thermometer until the first trace of opacity makes its appearance, generally as a fine tail in the fluid at the extremity of the thermometer's bulb. This is the required temperature. The glycerides of the saturated fatty acids are deposited as the acetic acid cools. The temperature corresponding with pure butter-fats runs from 29° C. to 39° C., and that for margarine falls between 94° C. and 97° C. A standard sample of butter may be tested against a weaker acid giving a temperature of turbidity of, say, 60° C. Margarine then gives 100° C. or over. This is a very good preliminary test for the differentiation of pure butters from margarine.

Examination under Polarized Light.—A small particle of butter is placed on a clean microscopic slide, and a cover-glass affixed. The slide is placed on the stage of a microscope provided with crossed nicols, and examined with a coarse objective. In order to shut out light from the upper surface a short black tube is laid on the slide in such a manner that the objective dips into it. When pure butter-fat, which is non-crystalline, is examined, it pre-

sents a uniformly dark field. On the other hand, when margarine is examined, certain portions of the field are bright, and crystalline masses are dimly perceived. These may be critically studied by uncrossing the nicols.

To Detect Cottonseed Oil in Butter.—To the melted fat add an equal volume of a saturated solution of lead acetate, and a smaller quantity of ammonia, and stir. On standing for a little time the superficial layers turn orange-red.

Bechi's Silver Test.—Dissolve 1 gramme AgNO_3 in 100 c.c. of 95 per cent. alcohol; add 20 c.c. ether and a drop of HNO_3 , and thoroughly mix. To 10 c.c. of the sample of fat add 2 c.c. of this reagent. Mix and stand the test-tube in boiling water for fifteen minutes. The mixture assumes a considerably darker tint, due to reduced silver, in the presence of cottonseed oil. These tests are best performed in the presence of blank tests on pure butter-fat.

Halphen's Test.—Mix equal volumes of amyl alcohol and CS_2 in which 1 per cent. S has been dissolved. To 5 c.c. of this mixture add an equal volume of the fat in a test-tube, and heat in a bath of boiling saturated brine for fifteen minutes. A deep red or orange colour is produced in the presence of cottonseed oil. In its absence little or no colour is developed. Pyridin in the amyl alcohol appears to be the active reagent.

Sesame Oil.—Fats containing this oil give a red colour when heated with stannous chloride on a water-bath. The colour is not discharged by moderate dilution with water, thereby differing from the colour produced by turmeric.

Baudouin's Test.—Dissolve 0.1 gramme cane-sugar in 10 c.c. HCl (specific gravity 1.2). Add to the solution in a test-tube 20 c.c. of the fat and shake thoroughly for a minute. Allow to stand till the oil separates from the aqueous solution. In the presence of 1 per cent. sesame oil the aqueous solution is coloured deep red.

Colouring Matters.—Annatto, turmeric, and some coal-tar products have been used to increase the yellow tint of butter. These colouring matters are, for the most part, vegetable, and harmless. If the colouring matter can be extracted with alcohol it is foreign, since the natural colouring matter of butter is not soluble in alcohol. Coal-tar dyes may be fixed on silk or wool by boiling fibres in the alcoholic extract diluted with water and acidified with HCl .

If saffron be present, the alcoholic extract will be coloured green by HNO_3 , and red by HCl and sugar.

Turmeric is detected by evaporating the alcoholic extract to dryness, and boiling the residue in a few c.c. of dilute boric acid solution. A strip of filter-paper soaked in the latter and slowly dried becomes cherry red. Addition of a drop of alkali turns the red to olive green.

In recent years a number of liquid fats have been hydrogenated by the catalytic action of nickel and other catalysts—oleic acid, *e.g.*, becoming stearic acid— $\text{C}_{18}\text{H}_{34}\text{O}_2 + \text{H}_2 = \text{C}_{18}\text{H}_{36}\text{O}_2$. The physical change from liquid to solid has enabled manufacturers to incorporate various oils in margarines and butter. Whether such hardened fats are equally digestible and equally nutritious with the natural bodies they now chemically represent remains to be seen. Their appearance has caused considerable trouble to analysts, as many of the physical and chemical constants have been completely upset.

Bacteria in Butter.—Economic bacteria take part in the conversion of cream into butter. In Europe and America much butter is made from pasteurized cream, to which 'starters' (cultures of lactic acid bacteria) are added. By this means the process of butter-making is much better controlled, and results are much more uniform. British butter contains from 1,000,000 to 50,000,000 micro-organisms per gramme. The bulk of these are *Bacillus acidilactici*, *B. lactis aerogenes*, etc., which keep in check the development of unfavourable bacteria, such as *B. mesentericus*, *B. fluorescens*, *B. subtilis*, etc., which give origin to evil flavours, bitter taste, and rancidity.

The principal pathogenic organism found in butter is the *B. tuberculosis*. To detect this organism warm a sample of butter to 42°C . Centrifugalize the liquid, and inoculate guinea-pigs with the sediment. It is of importance to note that the butter bacillus of Rabinowitsch and Petri is acid-fast, and morphologically like the tubercle bacillus; and, moreover, when injected intraperitoneally mixed with butter, it produces similar lesions in guinea-pigs. It is, however, readily distinguished from *B. tuberculosis* by its rapid growth on glycerine agar and other ordinary media, forming an abundant dry mass in three or four days.

CHEESE

Cheese consists, for the most part, of proteins and fat. It may be prepared (1) by adding rennet to milk, whereby the casein clots and entangles most of the fat; and (2) by allowing the milk to become sour through the formation of lactic acid, or by the addition of a dilute acid, such as vinegar, when the cheese contains little fat.

The characters of different cheeses depend on the kinds of milk used, the methods of preparation employed, and the types of micro-organism admitted to the original milk or to the cheese whilst ripening. During the ripening of cheese a partial digestion of proteins is effected, resulting in the production of the so-called primary products of digestion—albumoses and peptones. Later, secondary products of ripening are found—viz., amido-compounds and ammonia.

Whether during these changes fat is increased at the expense of protein, as was once believed, is doubtful. The relative proportions of this digestive work carried out respectively by milk enzymes and by enzymes of added bacteria are unknown. The flavour of a particular cheese is due to the micro-organisms growing in it during ripening. The old idea that a particular cheese, such as Stilton, can be made only in one locality is exploded. Magnificent Stiltons are now made in Hampshire by the agency of a 'cheese mould' carried to that county from Leicester.

Soft cheeses, such as Brie and Camembert, are produced by clotting milk with rennet at temperatures below 30° C., and using little pressure. Hard cheeses, like Stilton, Cheddar, Gorgonzola, and Gruyère, are clotted at higher temperatures—30° to 35° C.—and submitted to greater pressure. Soft cheeses contain much water, and therefore fail to keep long.

The nitrogen of the proteins in cheese exists in a variety of forms. Van Slyke found that the 3.86 per cent. N of an American cheddar was distributed as follows: Water-soluble N 1.46, paracasein-mono-lactate 0.94, paranuclein 0.14, caseoses 0.22, peptones 0.18, amides 0.79, and ammonia 0.13.

A full-cream cheese contains 30 to 35 per cent. butter-fats. Filled cheeses may contain any proportions of foreign fats mixed with butter-fats.

If the fat is considerably less than the protein, the cheese was made from skimmed milk.

In a whole-milk cheese the ratio $\frac{\text{fat}}{6.37 \text{ total N}}$ is greater than 1 (generally 1.25 to 1.5). In a skimmed-milk cheese this ratio is less than 1.

The digestibility of cheese in the stomach is less than that of meat, on account of its proteins being covered with fat. Cheese should therefore be well masticated, or, better, thoroughly grated before being used. Its digestion in the small intestine is effected without difficulty. Owing to the small quantity of water contained in cheese compared with that in beef, it has a higher nutritive value than the latter. The energy derivable from cheese, as measured by calories, is about three times that of beef. Moreover, the fact that the protein of cheese is chiefly casein, and accordingly purin-free, should highly recommend it as an article of diet to those who are in any way troubled by uric acid.

Percentage Composition of a Few Soft Cheeses :

| | | | Water. | Proteins. | Fat. | Ash. |
|---------------|----|----|--------|-----------|------|------|
| Camembert | .. | .. | 50.9 | 18.6 | 27.4 | 3.1 |
| Brie .. | .. | .. | 50.0 | 18.3 | 27.6 | 4.1 |
| Stracchino .. | .. | .. | 39.2 | 29.3 | 27.7 | 3.8 |

Percentage Composition of a Few Hard Cheeses :

| | | | Water. | Proteins. | Fat. | Ash. |
|----------|----|----|--------|-----------|------|------|
| Cheddar | .. | .. | 27.2 | 36.6 | 32.0 | 4.2 |
| Cheshire | .. | .. | 30.4 | 36.1 | 28.7 | 4.8 |
| Stilton | .. | .. | 28.6 | 35.6 | 31.8 | 4.0 |
| Gruyère | .. | .. | 32.0 | 35.1 | 28.1 | 4.8 |

The ripening of cheese has been somewhat differently explained by Freudenreich, Duclaux, and Babcock and Russell. When the curd is thrown down by rennet, it carries with it most of the bacteria of the milk. Freudenreich believes that the lactic acid organisms, which develop early and rapidly, are the chief factors in the process of ripening. Duclaux holds that, since the ripening proceeds after the lactic acid organisms have considerably diminished, the active agents are enzymes secreted by a variety of organisms, which he

names *Tyrothrix*. Babcock and Russell accept the view that ripening is effected by an enzyme originally present in milk.

Moulds in Cheese.—Green mould (*Penicillium glaucum*) is found in Roquefort and Gorgonzola. *Aspergillus glaucus* produces the appearance known as blue mould, whilst red mould is accounted for by the growth of *Sporendonema casei*. The common mould (*Mucor mucedo*) is found in more than one variety.

Of animal parasites found in cheese, the two most frequently met with are 'the cheese mite' (*Acarus domesticus*), and 'cheese maggots' (larvæ of *Piophilæ casei*).

Adulteration in Cheese.—The principal adulterations in cheese are the use of skimmed milk for whole milk, and the addition to skimmed milk of foreign fats. Mineral adulterants, such as chromate of lead, used to tint the rind and sulphate of zinc ('cheese spice'), used to prevent gas formation from fermentation, are rarely met with.

Estimation of Water in Cheese.—Dry 5 grammes cut into thin slices in an air oven at 100° C. to constant weight. Loss of weight equals water.

Ash.—Ignite the residue from the water determination at a low red heat; cool in a desiccator and weigh.

Fat.—Place 50 grammes of cheese in a muslin bag in a beaker on a water-bath; the fat will pass out in a pure state into the beaker. Perform the Valenta and Reichert-Meissl tests on this (as described under Milk) to detect and estimate amounts of pure butter-fats and foreign fats.

The total fat is estimated as follows: Grind 5 grammes of cheese in a mortar with 10 grammes of anhydrous copper sulphate; place a layer of anhydrous copper sulphate about 2 centimetres thick on the bottom of the receiver of a Soxhlet; add the ground mixture, and rinse the mortar with a little of the ground sulphate and afterwards with ether. Extract for sixteen hours. Evaporate the ether from the extraction flask, and dry the fat in a steam-chest to constant weight.

Werner-Schmidt Method.—Boil 2 grammes of cheese with 5 c.c. of water and 10 c.c. of concentrated HCl in a large test-tube, with constant shaking until all but the fat is dissolved. Cool; add 25 c.c. ether, and shake well. When separated, draw off as much

as possible of the ether. Extract with four additional portions of ether, and collect the whole in a flask. Distil off the ether and weigh the fat.

Proteins.—Treat 1 gramme of cheese by the Kjeldahl method. $N \times 6.25 = \text{proteins}$.

Separation and Determination of N Compounds (Van Slyke).

Mix 25 grammes of cheese with an equal weight of quartz sand in a mortar, and transfer to a flask; add about 100 c.c. of water at 50° , and keep the temperature at 50° to 55° for half an hour, shaking frequently the while. Decant the liquid through a cotton filter into a 500 c.c. graduated flask. Treat the residue with repeated portions of 100 c.c. of water in the same manner until the water extract amounts to just 500 c.c. Employ aliquot parts of this for the various estimations.

Water-Soluble N.—Perform the Kjeldahl process on 50 c.c. of the water extract ($= 2.5$ grammes of cheese).

Para-Nuclein N.—To 100 c.c. water extract add 5 c.c. of a 1 per cent. HCl solution; stand at 50° to 55° till separation is complete, as shown by a clear supernatant liquid. Filter, wash the precipitate with water, and determine the N by Kjeldahl.

N as Coagulable Protein.—Neutralize the filtrate from the last determination with dilute KOH; heat at 100° till the coagulum, if any, settles out completely. Filter, wash the precipitate, and determine the N in it as above.

N as Caseoses.—Treat the filtrate from the preceding with 1 c.c. of 50 per cent. sulphuric acid, saturated with $ZnSO_4$, and warm to 65° to 70° until the caseoses settle out completely. Cool, filter, wash with saturated $ZnSO_4$ acidified with H_2SO_4 , and determine the N in the precipitate.

N as Amides and Peptones.—Put 100 c.c. of water extract in a 250 c.c. graduated flask, add 1 gramme NaCl and a 12 per cent. solution of tannin till a drop added to the clear supernatant solution fails to produce further precipitation; dilute to the mark, shake, and pour on a dry filter; determine the N in 50 c.c. of the filtrate = N in amido-acid and ammonia compounds. This minus the ammonia N = amido-N. Peptone N = total N in water extract minus sum of para-nuclein N, coagulable protein N, and N of caseoses, amides, and ammonia.

N as Ammonia.—Distil 100 c.c. of the filtrate from the tannin-salt precipitation into standard acid, and titrate against standard alkali.

N as Para-Casein Lactate.—Wash the insoluble residue produced in obtaining the water extract with several portions of a 5 per cent. NaCl solution to form a 500 c.c. salt extract; determine the N in an aliquot part of this salt extract.

Determination of Lactose.—Boil 25 grammes of finely divided cheese with two portions of 100 c.c. each water. Pour on filter, wash residue with hot water, make up the watery extract to 250 c.c., and determine the lactose by the Fehling or Pavy-Fehling method.

Detection of Foreign Fat.—Submit the prepared fat to the Reichert-Meissl method.

Detection of Bacillus Tuberculosis.—Rub up portions from the central parts of the cheese with sterile normal saline until a good emulsion is obtained. Strain through sterile absorbent cotton, and inject the equivalent of 2 grammes of cheese into each of two or three guinea-pigs.

Lard.—Freshly rendered lard (internal abdominal fat of pig) contains no free fatty acids. It is much adulterated with cottonseed oil and beef stearin. It has the following constants: Melting-point, 36° to 45° C.; iodine absorption, 50 to 65 per cent.; saponification value, 195 to 197; Zeiss butyro-refractometer at 40° C. = 48.8° to 51°; specific gravity at 15.5° C., 0.931 to 0.932.

If the iodine value fall outside the above limits, the lard is adulterated, but a normal iodine figure is no guarantee of genuineness, as a judicious mixture of cottonseed arachis or other oil, with beef stearin, will give normal values when tested.

Infants' Foods.—The market is flooded with a large number of products of very varying composition. If the milk preparations (condensed, dried, and humanized milks) be grouped as a class, all the other foods contain flours in which the starch is altered or unaltered, or capable of being altered or otherwise during preparation of the food. It is necessary to determine the presence, nature, and amount of unaltered starch, the extent to which the starch is converted during the preparation of the food according to instructions on the label, the presence or absence of diastase in active form, and the nature of the cereal from which the starch is derived.

With the exception of full-cream dried milks and full-cream condensed milks, it may be fairly stated that practically all the infants' foods advertised are highly deficient in fat, and many deficient in proteins.

The only physiologically suitable food for a young mammal is the milk of its mother or some other animal of the same species.

The chemical composition of a foodstuff is no criterion of its nutritional value. Due proportion of protein carbohydrate and fat does not constitute a correct diet. For example, the proteins of different milks vary because of the fact that milks have a developmental as well as nutritive function: accordingly milks of different species are not interchangeable. It is known that the milk of animals whose chief digestion is gastric (cow, goat, etc.) forms solid clots of casein, whilst that of animals whose chief digestion is intestinal (mare, etc.) does not form solid clots, but soft gelatinous masses, which easily traverse the stomach and intestine.

The digestion of infants is largely intestinal, and human milk is the only form which in the early days puts no strain on it. It is common clinical knowledge that infants in the first months of life fed on artificial foods containing starch become the subjects of scurvy, atrophy, and gastro-intestinal disorders. The process of digestion is accompanied by the liberation of considerable potential energy, and in the case of the infant with little energy to lose the digestion of an artificial food, containing in addition to a foreign milk starch only partially converted, there may not be nearly sufficient energy to meet the largely increased call, with the well-known accompaniments of this failure—grave nutritional disturbance, rachitis, scurvy, anæmia, and more than one form of profound gastro-intestinal fermentation.

Estimation of Starch: 1. Direct Conversion by Acid.—Hemicellulose and all carbohydrates capable of conversion to sugar are included with starch.

Wash 2 grammes of the finely divided material on a filter with ether, using 10 c.c. four or five times; continue the washing with first 100 c.c. 10 per cent. alcohol, and then with 10 c.c. absolute alcohol. Now wash off the contents of the filter into a flask with 150 c.c. water and 20 c.c. HCl (specific gravity, 1.125). Place the flask on a boiling-water bath under a reflux condenser for two hours.

Cool, neutralize with NaOH, add alumina cream if necessary, mix, make up to $\frac{1}{2}$ litre, filter, and estimate the dextrose in an aliquot part of the filtrate by the polarimeter or by Fehling's method. Calculate the dextrose figure into starch by multiplying it by 0.9.

2. Conversion by Diastase.—By this method starch only is acted on; hence in the presence of other substances it is to be recommended. Starch is first converted into maltose and dextrin, and finally into glucose.

Prepare 2 grammes with ether and alcohol as above. Wash off the filter into a beaker and boil for fifteen minutes, or until completely gelatinized, stirring constantly. Cool to 55° , and add sufficient malt extract (10, 15, 20 c.c., according to degree of activity), or better animal diastase, and digest for an hour at 55° . Boil for fifteen minutes, add further animal diastase, replace water lost by evaporation, and digest for another hour, or until when treated with iodine under the microscope no starch appears. Cool, make up to 250 c.c., add 20 c.c. HCl (specific gravity, 1.125), and proceed as in the acid conversion method.

Reducing Sugars as Dextrose (Lactose excepted).—A cold-water extract is made and titrated with Fehling's solution.

Lactose.—Baker and Hulton have shown that an aqueous solution of lactose, unlike maltose, dextrose, cane-sugar, etc., is not fermented by ordinary brewer's yeast; hence small amounts of lactose added to flours, etc., can be estimated by measuring the reducing action on Fehling's solution of the residue after fermentation.

Boil the aqueous extract with 2 per cent. of citric acid to invert any cane-sugar, thus facilitating fermentation; neutralize; cool and add a little cold aqueous extract of diastatic malt. Close the containing flask with cotton-wool, and incubate at 27° C. for seventy-two hours. The solution, now destitute of all reducing sugars except lactose, is cleared with alumina cream, filtered, boiled, made up to an appropriate volume, and titrated with Fehling's solution. Ten c.c. of Fehling's solution = 0.074 gramme of pure lactose.

Cane-Sugar.—A portion of cold water extract is boiled as above with 2 per cent. citric acid, the solution neutralized, and titrated with Fehling's solution. From the value of the invert sugar so

obtained is subtracted the dextrose already found; the difference reduced by 5 per cent. (hydration correction) is the percentage of cane-sugar.

Fat.—Fat of dried milks. Owing to the inclusion of fat globules amongst dried proteins the solvent action of ether in the Soxhlet method may be greatly inhibited. The Werner-Schmidt method is in this case more suitable.

Proteins.—The N is determined in 0.5 gramme of the food by Kjeldahl's method, and the figure $\times 6.25$.

Water.—Five grammes are dried on a water-bath (five hours or longer) to constant weight.

Ash.—Five grammes are burnt at a dull red heat in a muffle. If the food burns with difficulty, H_2SO_4 may be added, and a correction made by deducting one-tenth of the weight of the ash.

Cellulose (material insoluble in boiling water and not attacked by diastase).—To 5 grammes of the food freed from fat by ether if necessary add 200 c.c. distilled water, and bring to the boil. Continue the boiling for half an hour. Add some cold extract of malt (15 to 25 c.c. according to degree of activity), and digest at 55° to 60° for three or four hours. Filter through a dry tared filter. Wash the residue repeatedly with water at 60°C . until free from all reducing sugar; then with alcohol and ether. Dry for several hours on water-oven and weigh. Transfer filter-paper and residue to a Kjeldahl flask, and determine the protein. Carry out the procedure in duplicate, but in the second estimation determine the ash instead of the protein.

The first weight less the protein and ash = cellulose.

Saccharifying Diastase.—Two or three c.c. of a 5 per cent. cold-water extract of the food are allowed to act for an hour at 21° on 100 c.c. of a 2 per cent. soluble starch solution. At the end of the hour the action is stopped by the addition of 10 c.c. $\frac{\text{N}}{10}$ NaOH, and the whole made up to 200 c.c. The amount of maltose present is determined by Fehling's solution. A food may be regarded as having a diastatic activity of 100 when 0.2 c.c. of the 5 per cent. solution produces under these conditions sufficient maltose (0.08 gramme) to reduce completely 10 c.c. Fehling's solution. If double the amount is required, the diastatic power is 50, etc.

CEREALS.

The composition of a few common cereals is given in the following table:

| | | Protein. | Fat. | Carbo-
hydrates. | Cellulose. | Water. | Ash. |
|-----------|----|----------|------|---------------------|------------|--------|------|
| Wheat | .. | 11.0 | 1.7 | 71.2 | 2.2 | 12.0 | 1.9 |
| Barley | .. | 10.1 | 1.9 | 69.5 | 3.8 | 12.3 | 2.4 |
| Rye .. | .. | 10.2 | 2.3 | 72.3 | 2.1 | 11.0 | 2.1 |
| Oats .. | .. | 11.0 | 5.2 | 57.3 | 12.0 | 11.8 | 2.7 |
| Maise .. | .. | 9.9 | 5.4 | 68.9 | 2.2 | 12.3 | 1.3 |
| Millet .. | .. | 10.4 | 3.9 | 68.3 | 2.9 | 12.3 | 2.2 |
| Rice .. | .. | 6.8 | 1.6 | 68.1 | 9.0 | 10.5 | 4.0 |

It will be seen that the proteins vary somewhat in amount in the different cereals. The fat appears in increased quantities in those cereals which grow in high latitudes. The chief carbohydrate is starch: it forms 65 to 70 per cent. of the whole grain. The ash averages about 2 per cent., and is composed principally of lime and phosphoric acid, thus resembling the ash of animal foodstuffs much more than that of vegetables. The high percentage of carbohydrates is an indication that cereals should be mixed with other foods richer in proteins and fat; this physiological requirement we find almost universally complied with: butter is spread upon bread, and the mixture eaten with cheese. On the whole, cooked cereals are easily digested and absorbed.

Wheat-Flour.—Wheat is the most important cereal used in this country. It is consumed to the extent of six bushels per head per annum. The grain of wheat consists of three portions: (1) The bran or outer envelope of cellulose, containing mineral matter, and forming 13.5 per cent. of the grain; (2) the endosperm, constituting 85 per cent. of the whole, and consisting of nutritive material for the growth of the embryo; (3) the embryo or young plant, forming 1.5 per cent. of the grain. The bran consists of an outer layer of fibres of cellulose impregnated with salts, a middle layer of pigment cells, and an inner layer of aleurone grains. The endosperm consists of a delicate reticulum of cellulose, in whose meshes are found numerous starch granules. The embryo is composed of small cells rich in protein and fat.

The milled grain known as flour differs in composition, according

to whether the bran or embryo, or both, have been largely removed or retained. The reduction of bran to a powder by grinding is a difficult and expensive matter, and as a rule the miller removes it altogether. In roller-milling, the germ is also removed, in order to prevent the fat which it contains becoming rancid. Enzymes present in the germ act upon the starch, converting it into dextrin and sugar, which darken the colour of the bread; so the germ is excluded. This rejection of the bran and germ means the loss of some of the most useful constituents of the wheat; and the recognition of this loss has led to a number of patent processes for treating the bran and germ so as to prevent the production of a dark loaf. In the 'Hovis' process, the fat of the germ is treated with steam, with the object of preventing its becoming rancid. In the 'Frame Food' process, the bran is boiled with water under pressure, with the object of breaking down the cellulose, and extracting the bulk of the nitrogenous and mineral constituents. In Smith's patent the germ is partially cooked by superheated steam, whereby the ferment is killed which transforms the starch of the flour. According to the method adopted in milling, some flours contain more bran than others, and some more starches and gluten.

Wheat from different countries varies in chemical composition. Ordinary bread is made from a mixture of flours derived from different wheats, and sometimes such a mixture includes different types of milling.

The average composition of wheat-flour is:

| | | | | | | | |
|--------------------------------|----|----|----|----|----|----|------|
| Water | .. | .. | .. | .. | .. | .. | 13·0 |
| Sugar | .. | .. | .. | .. | .. | .. | 0·7 |
| Ash | .. | .. | .. | .. | .. | .. | 0·8 |
| Fat | .. | .. | .. | .. | .. | .. | 1·5 |
| Protein | .. | .. | .. | .. | .. | .. | 11·0 |
| Starch, dextrin, and cellulose | .. | .. | .. | .. | .. | .. | 73·0 |

Physical Characters of Flour.—Flour should be free from acidity, white in colour, and smooth when rubbed between the fingers. It should be entirely free from fungi and all other parasites. A yellow colour denotes age or fermentation. If flour be kept in a damp place, an odour is generated by the growth of moulds and various micro-organisms.

Gluten.—The crude protein of flour known as gluten possesses

a constituent, gliadin, which confers upon dough its characteristic adhesiveness. When dough is thoroughly washed so as to get rid of starch and all other soluble bodies, such as salts, albumin, sugar, etc., gluten remains as a somewhat tough and sticky mass; when this is blown up with a gas, it coheres sufficiently to remain in the form of a sponge. Barley, rice, and oatmeal do not contain gluten, but other forms of protein, which are destitute of this viscid character; hence they cannot be made into bread unless mixed with a sufficient quantity of wheat-flour.

Estimation of Gluten.—Place 20 grammes of flour in a basin and stir it into a stiff dough with warm water; next thoroughly work the dough with the fingers in a fine muslin bag in a stream of running water until all the starch and other soluble materials have been washed away. The absence of starch may be proved by the iodine test. Generally a small quantity of fats and salts (1 per cent.) remains. Now spread out the gluten in a weighed dish in a water-oven; dry until a constant weight is obtained. This weight, minus the weight of the dish, represents the gluten.

A more delicate and reliable method is the estimation of total nitrogen by Kjeldahl's process, as described previously. The total $N \times 6.3$ gives approximately the gluten. In carrying out the process great care should be taken that no ammonia and no nitrates exist in the reagents used. If the gluten fall below 8 per cent., the flour may be regarded as not pure wheat-flour.

Ash.—The ash of wheat-flour consists principally of phosphates of potassium, magnesium, and calcium, together with mixed salts of sodium and iron, and lastly silica. The total quantity should not much exceed 1 per cent. The estimation should be done in a platinum basin, and a wholly white ash obtained. Ash amounting to 2 per cent. shows the addition of mineral adulterants.

Water.—This constituent should not exceed 16 per cent. The adulteration of wheat-flour at present consists essentially in the addition of other flours, as those of rice, maize, pea, and bean. The microscopic appearances of the different starch granules will assist in the detection of such adulterations.

Starch Granules.—To estimate the amount of starch in a substance, weigh out a gramme of the dried powdered material, and mix it with 50 c.c. of a 5 per cent. HCl solution in a flask, to which a

reflex condenser is attached; boil for several hours under a hood: the starch is converted into sugar (dextrose). Make the solution slightly alkaline with NaOH solution, and estimate the dextrose by Fehling's method. The result, multiplied by 0.9, gives the quantity of starch in a gramme. Where cellulose is present, the small amount converted into sugar may be ignored. The microscopic appearances of many starch granules are such as to afford an easy means of recognition. If a mere speck of a particular flour or powdered starch be placed on a microscopic slide, a drop of water added, and a cover-slip applied, the starch granules can be thoroughly studied by low and high powers of the microscope. As in mounting specimens of bacteria, it should be noted that it is almost impossible to apply too little of the material to the slide. The student should observe that in most cases characteristic cells appear, but that many cells may be unrecognizable, as belonging to any particular kind of starch. Where starch granules of different foodstuffs closely resemble each other, it may be quite impossible to decide whether or not slight admixture has been effected. On the other hand, when the granules are dissimilar the slightest admixture is easily detected.

If an estimation of the amount of the adulteration be required, a rough average percentage of the foreign granules may be obtained by counting a number of fields, and this estimation may be checked by making a mixture containing the true and foreign ingredients in the proportions observed; such mixture should present the same microscopic appearances as the original. Several trials may be made in this way before the required match is obtained. The student should carefully study the microscopic characters of all starch granules occurring in vegetable foods, and make drawings of them.

Bleaching of Flour and Flour-Improvers.—With a view to improving the baking qualities of flour, millers resort to bleaching and the addition of 'improvers.' Ozone, halogens, and nitrogen peroxide have been used as bleaching agents. Nitrogen peroxide alone appears to give satisfactory results, and is the only bleacher now used. The gas is produced chemically from nitric acid and ferrous sulphate, or electrically by the combination of the N and O of the air by an electrical sparking discharge. The latter is said

to be the better method in that the degree of bleaching is more easily controlled, and condensation of acid resulting in staining of the flour less likely to occur. Air charged with nitrogen peroxide and ozone is agitated with the flour in a suitable machine. It is stated that the nitrogen peroxide produced by 3 c.c. of nitric oxide in 3 litres of air will bleach 1 kilogramme of flour.

A watery extract of bleached flour reacts to the nitrite test of Griess. This reaction is not given by ordinary unbleached flour.

When bleached flour is baked, one-half to two-thirds of the nitrite disappears, and an increase in nitrates occurs. The whole of the nitrite may disappear from biscuits.

Effects of Bleaching.—Bleaching destroys the yellow colouring-matter dissolved in a thin layer of oil which surrounds the individual granules of starch; the iodine value of this oil is lowered. The acidity of flour is increased. It is probable that certain amino-groups in the protein are destroyed.

Improvers used.—Water added to the flour in a fine spray; phosphates, especially calcium phosphate; phosphoric acid; sulphuryl chloride. It has been experimentally shown that even traces of nitrites in flour inhibit both proteolytic and amylolytic digestion.

The introduction of roller-milling made it possible to utilize any variety of wheat since pulverization of the bran is avoided, and consequently a more complete removal of bran and germ effected.

The germ contains no gliadin nor glutenin (these substances unite with water to form gluten); it contains 10 per cent. of albumin, 5 per cent. of globulin, and 3 per cent. of proteose. Its nucleated cells contain a considerable amount of nucleic acid combined with albumin and globulin. Little organic phosphorus accordingly occurs in the endosperm. Wheat contains probably about 2 per cent. of germ, and as the latter possesses at least 30 per cent. of proteins, retention of the germ raises the protein content of flour by 0.6 per cent.

The greater portion of the phosphorus in bran can be extracted with dilute acid, and it has been shown that the bulk of this phosphorus occurs in organic combination as a phospho-organic acid, combined with potassium, calcium, and magnesium.

Wholemeal or Graham flour is produced by grinding the entire wheat grain; it should contain the whole of the germ.

'Entire' wheat flour or fine meal is obtained by removing a portion of the bran, and finely grinding the rest; it contains a portion of the germ.

'Straight-run' flour is the whole of the flour produced in the roller-mill. The percentage composition of bread made from samples of these flours is as follows:

| | Protein
(N \times 5.7). | Carbo-
hydrates. | Fats. | Water. | Ash. |
|-------------------|------------------------------|---------------------|-------|--------|------|
| Graham flour .. | 9.54 | 46.10 | 0.29 | 42.68 | 1.39 |
| 'Entire' .. | 9.32 | 48.75 | 0.19 | 40.97 | 0.77 |
| 'Straight run' .. | 9.63 | 51.06 | 0.04 | 38.77 | 0.50 |

Experiments have been made on the nutritional values of different varieties of flours. Young rats have been fed on 'standard' or 'straight run,' and others of the same age on 'entire.' The first lot thrived much better than the second. Again, the same experiment has been carried out with Graham flour and 'entire,' or white flour, with results in favour of the Graham variety.

In the milling of rice the cuticle, consisting of pericarp, testa, and nucellus, is frequently removed. A diet consisting exclusively of such rice produces polyneuritis and other changes, constituting a disease known as 'beri-beri.' If the offal (about 10 per cent. of the grain) be returned to the rice, no beri-beri occurs. Or if the offal be extracted by 0.3 per cent. HCl and the extract precipitated by proof spirit, the substances soluble in alcohol (1.6 per cent. of the grain) will equally prevent the disease. It is significant that the precipitate containing 85 per cent. of the phosphorus of the offal is wholly ineffective in preventing the disease. A good rice should not contain less than 0.4 to 0.5 per cent. total phosphorus. The milling of rice deprives it of its cuticle, and leaves it with a dull appearance. To improve its appearance it is 'polished' in hollow cylinders fitted with revolving rollers covered with sheep-skin. In order to obtain a high polish talc or steatite, in the form of a fine powder, is added to the rice prior to polishing, and for the most part as it passes through the mill. Gypsum, kaolin, and gums have been also used for this purpose. The colour of rice is changed from a cream to a dead white by the addition of blue pigments (generally ultramarine) during milling, and to make it transparent it is treated with arachis and other oils.

1. **Granules of Wheat, Barley, and Rye.**—*Wheat.*—These are (1) large, round, or oval, which do not exhibit concentric striæ;



FIG. 29.—WHEAT. $\times 200$.

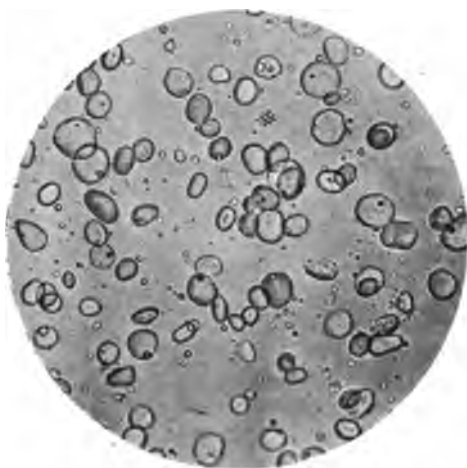


FIG. 30.—BARLEY. $\times 200$.

(2) small, ill-defined granules scattered irregularly throughout the field. Intermediate sizes are rare.

Barley.—These are (1) large, (2) small, (3) intermediate in size. In a very few are there any markings.



FIG. 31.—RYE. $\times 200$.

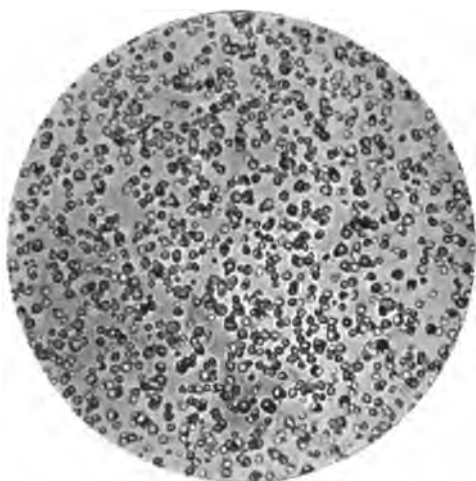


FIG. 32.—RICE. $\times 200$.

Rye.—These are very similar to those of barley, except that in the large granules some show a rayed hilum and cracked edges;

the large granules are more generally circular and flattened than those of wheat and barley, and somewhat larger.



FIG. 33.—OAT. $\times 200$.

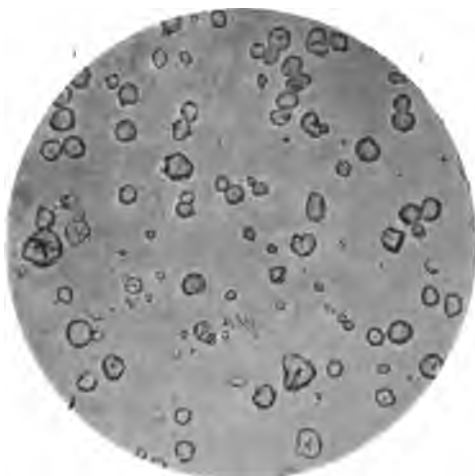


FIG. 34.—MAIZE. $\times 200$.

2. **Rice, Oatmeal, and Maize** exhibit small faceted and angular granules destitute of concentric markings. The granules

of rice are small, and collect in part into angular masses. Those of oatmeal are slightly larger, and collect into rounded masses.



FIG. 35.—SAGO. $\times 200$.



FIG. 36.—TAPIOCA. $\times 200$.

Maize granules are much larger and more irregular in shape, and most of them possess a stellate hilum.

3. **Sago and Tapioca**—These granules are irregular in outline, being angular and partially rounded; they are irregular in



FIG. 37.—PEA. $\times 200$.

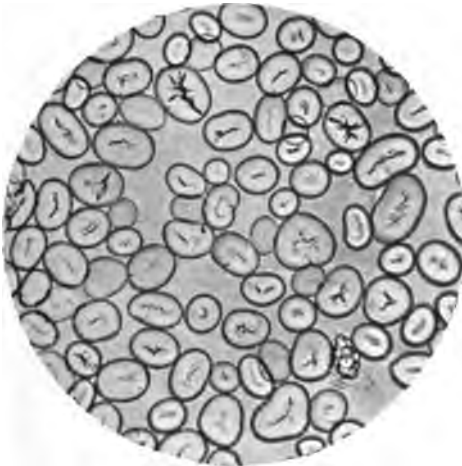


FIG. 38.—HARICOT BEAN. $\times 200$.

size, and mostly possess a central hilum; occasionally they exhibit concentric striæ. Sago granules are large and very irregular in

shape—for the most part somewhat rounded at one side and truncated at the opposite; the hilum is either stellate or linear.



FIG. 39.—ARROWROOT. $\times 200$.



FIG. 40.—POTATO. $\times 200$.

Tapioca granules are much smaller, and the hilum is generally placed towards the rounded extremity.

4. **Pea and Bean.**—These granules are oval in form, fairly uniform in size, and possess a central linear hilum and faint concentric striæ. Those of the pea present a central longitudinal hilum, sometimes exhibiting cross-striation. The granules of the bean are somewhat larger and broader, and the cross-striation of the central hilum is more marked.



FIG. 41.—*VIBRIO TRITICI*. $\times 30$.



FIG. 42.—*BRUCHUS PISI*.



FIG. 43.—*ACARUS FARINÆ*. $\times 1$

5. **Arrowroot and Potato.**—The granules of these starches are large, pyriform, and marked distinctly with concentric striæ. A circular hilum is found in both, placed at the large extremity in arrowroot, and at the small in potato. The granules of arrowroot do not swell in a solution of KOH, as do those of the potato.

Parasites found in Wheat and Flour—*Animal: Tylenchus tritici* (ear cockle).—In the infected ears of grain are to be seen the larvæ of a nematode worm, occurring as a white powder in dark misshapen grains. Specimens may be mounted directly in

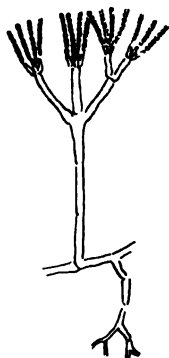


FIG. 44.—*PENICILLIUM GLAUCUM*.



FIG. 45.—*ASPERGILLUS GLAUCUS*.

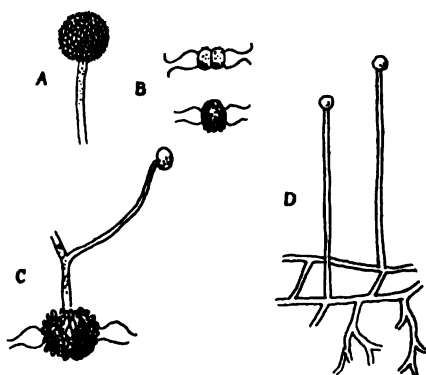


FIG. 46.—*MUCOR MUCEDO*.

A, Head; B and C, conjugation;
D, spore-bearing hyphæ.

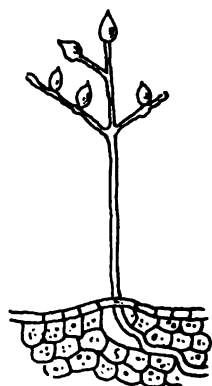


FIG. 47.—*PERONOSPORA*.

Farrant's solution, or dehydrated and cleared in the ordinary manner, and mounted in Canada balsam.

Bruchus pisi and *Calandra granaria* are beetles (Coleoptera) which infest grain and pulses. The first attacks the pea, an allied

species the bean, whilst *Calandra* is found in grain. The female lays her eggs in the young fruit, and the larvæ destroy the internal parts. The *C. granaria* perforates the husk of the grain and abstracts the contents.

Acarus Farinæ.—This parasite is found in inferior and damp flour. It may be distinguished from the *A. scabei* by its legs remaining thick up to their extremities, whilst in the itch parasite the distal ends of the legs are quite thin.

Vegetable Parasites found in Wheat, Flour, Bread, etc.

—**Moulds:** *Penicillium glaucum*, *Aspergillus glaucus*, *Mucor*



FIG. 48.—*USTILAGO SEGETUM*. X 250.

mucedo, and *Peronospora*.—These moulds are easily distinguished by the characters of the ends of the spore-bearing hyphæ. In *penicillium* the last hypha branches into three or four terminal filaments, which develop round or oval spores in rows in their long axes. In *aspergillus* the end of the spore-bearing hypha enlarges, and from this pedicle-bearing spores grow out and form a more or less dense head. In *mucor* the end of the spore-bearing hypha enlarges greatly, and the spores, instead of growing out from the enlargement, as in *aspergillus*, grow inside a membrane which surrounds the head. When the spores have matured, the membrane ruptures and sets them free.

Peronospora, which caused the Irish potato famine of 1847, first affects the leaves, then travels down the stem, and finally attacks the tubers. The spore-bearing hyphæ branch and rebranch, and at the end of a terminal branch a single spore is developed.

Ustilago Segetum (smut).—The spores of this parasite are found as a black powder infesting ill-developed ears of corn, and fall off when the ear is rubbed. Examined microscopically, they are seen to be brown, spherical, free spores.

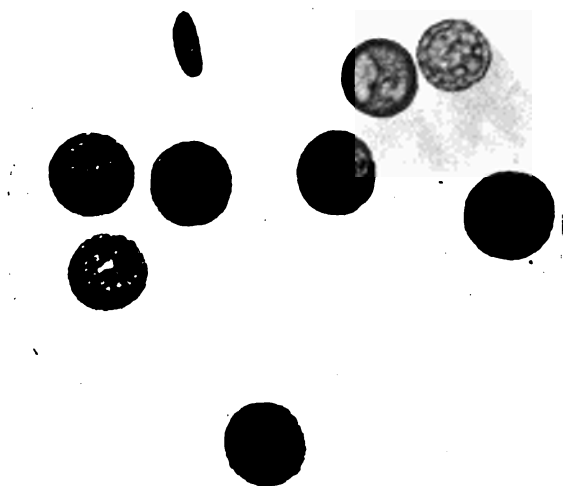


FIG. 49.—TILLETIA CARIES (UREDO FÆTIDA). $\times 250$.

Tilletia caries (bunt) is another member of the ustilaginae, and is found in the interior of the grain; it may escape detection until the process of milling takes place. The spores are brown, spherical bodies, generally free, and give to the interior of the affected grain a sooty appearance and foetid odour. These spores germinate in the spring, forming a hypha, the promycelium, which bears promycelial thread-like spores. The next stage in the life-history of this organism is the conjugation of contiguous spores. Two such conjugated spores bud and form an elongated secondary

promycelial spore, which, if it find a suitable host, sends out hyphæ and enters the interior of the grain, where a mycelium is developed. After a time the hyphæ swell, become dark in colour, and a differentiation into spores takes place. As these ripen, the mycelial structure disappears, and leaves the resting spores in the condition from which the cycle commenced.

Puccinia graminis is one of a large number of parasitic species affecting corn in the manner above described. A spore attaches itself to a grain or stem, and sends hyphæ into its substance, from



FIG. 50.—WHEAT STEM INFECTED WITH PUCCINIA.

which a mycelium and spores are formed within; as a result, the grain ruptures, and the spores appear on the surface as rust. A distinctive feature of puccinia is the double spore attached to a peduncle. It is this form which is found attached to grain or grass in the autumn, as rust, and which, known by the name teleuto-spore, remains quiescent during winter. In spring it germinates, and produces a non-parasitic mycelium. The individual cells of this mycelium produce filaments, known as gonidiophores, which in turn produce spores at their free ends. Distributed by the wind, these latter fall on the leaves of the barberry, where they

germinate and form a dense mycelium in the substance of the leaf, giving rise to swellings which project on its under-surface. Spherical



FIG. 51.—PORTION OF FIG. 50 SLIGHTLY MORE HIGHLY MAGNIFIED.



FIG. 52.—TELEUTOSPORES

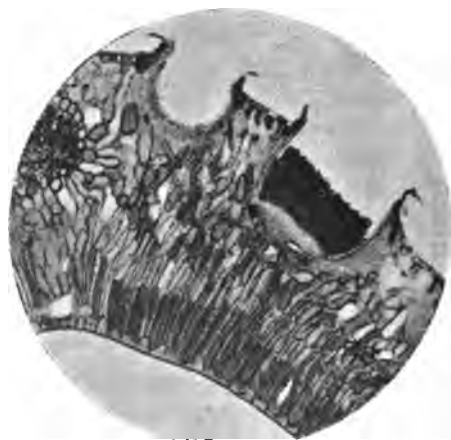


FIG. 53.—ÆCIDIUM BERBERIDIS.

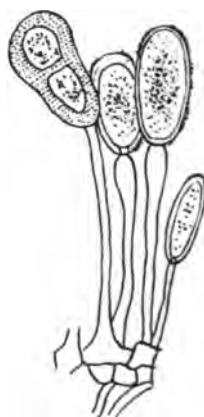


FIG. 54.—GONIDIOSPORES (UREDONGONIDIA) AND TELEUTOSPORE.

structures, termed æcidia, form and develop within themselves spores which are set free on rupture of the wall of the æcidium.

These spores are carried by the wind to grass plants, to which they attach themselves and develop a mycelium from which grow certain hyphæ, bearing single spores (uredogonidia). On rupture of the leaf of the host, the spores are to be seen as a yellow dust. Again the wind carries the gonidium to another grass, where it germinates, produces hyphæ, and repeats the previous process (uredo form). As the autumn approaches, special hyphæ produce gonidia, which have a septum perpendicular to the long axis, dividing the spore into two cells—the teleutospore; this rests



FIG. 55.—ERGOT IN RYE. $\times 30$.

through the winter, and commences the cycle once more in the following spring.

Claviceps purpurea (ergot) grows in rye, and the mycelial growth (sclerotium) replaces the grain. The ergot masses (or grains) are larger than the rye-grains, and of a deep purple colour. In the spring the sclerotium, which has rested through the winter, germinates, and produces long hyphæ (stromata), which develop a swelling at the distal end, which latter contains oval receptacles (ascocarps). Attached to the inner end of the ascocarps are asci, containing eight filiform spores (ascospores). The asci rupture

and the spores escape. Carried by the wind, the spores alight on the ovary of the rye flower and form a mycelium. On the surface of this mycelium free spores (gonidia) develop, and are surrounded by a viscid substance, known as honey-dew, which attracts insects,



FIG. 56.—SCLEROTIUM BEARING STROMATA. $\times 1$.



FIG. 57.—STROMA CONTAINING ASCOCARPS. $\times 75$

by which the spores are carried to other flowers, where the process is repeated. This stage is known as the sphacelia form.

As the rye is developed, the mycelial growth increases to such



FIG. 58.—ASCOCARP CONTAINING ASCI. $\times 350$.



FIG. 59.—ASCUS CONTAINING ASCOSPORES.

an extent that the young grain is wholly absorbed, the pericarp is no longer able to contain it, and it projects like a spur from the spike. Ultimately it falls to the ground. The cycle is repeated in the following spring.

The seeds of *Lolium temulentum*, possessing narcotic properties, may gain access to flour, but rarely produce poisoning.

BREAD

Bread is chiefly made from wheat-flour. A dough is first formed by mixing the flour with water or other fluid, and a gas, generally CO₂, is passed through it. Carbon dioxide is obtained either by the action of yeast on sugar, when this gas and alcohol are formed, or through the liberation of the gas from an alkaline bicarbonate by the action of an organic acid. The dough is sometimes aerated by charging water with air and mixing this with flour under pressure in air-tight chambers; afterwards the pressure is lowered by opening a trap, when the dough is blown up by the expanding gas, forming 'aerated' bread. The dough is then cooked in an oven at a temperature of 200-205° C.

Composition of White Bread:

| | | | | | | | |
|------------------------|----|----|----|----|----|----|------|
| Water | .. | .. | .. | .. | .. | .. | 40.0 |
| Proteins | .. | .. | .. | .. | .. | .. | 6.5 |
| Fat | .. | .. | .. | .. | .. | .. | 1.0 |
| Starch, sugar, dextrin | .. | .. | .. | .. | .. | .. | 51.2 |
| Cellulose | .. | .. | .. | .. | .. | .. | 0.3 |
| Ash | .. | .. | .. | .. | .. | .. | 1.0 |

Composition of Whole Meal:

| | | | | | | | |
|------------------------|----|----|----|----|----|----|------|
| Water | .. | .. | .. | .. | .. | .. | 45.0 |
| Proteins | .. | .. | .. | .. | .. | .. | 6.3 |
| Fat | .. | .. | .. | .. | .. | .. | 1.2 |
| Starch, sugar, dextrin | .. | .. | .. | .. | .. | .. | 44.8 |
| Cellulose | .. | .. | .. | .. | .. | .. | 1.5 |
| Ash | .. | .. | .. | .. | .. | .. | 1.2 |

During the cooking a crust is formed, which should neither be very light nor very dark in colour, and which should crack readily on breaking. The shining appearance of crust is due to the formation of dextrin, and its flavour and dark colour to the production of caramel. Two-thirds of the volume of a good loaf is gas. Great whiteness in a loaf, although much desired by the public, is by no means essential from a nutritive point of view, as a very white loaf possesses a maximum of starch and a minimum of protein.

Comparative Composition of Crust and Crumb:

| | Crust. | | | | | Crumb. |
|---------------------------|--------|----|----|----|----|--------|
| Water | .. | .. | .. | .. | .. | 44·45 |
| Insoluble protein | .. | .. | .. | .. | .. | 5·92 |
| Soluble protein | .. | .. | .. | .. | .. | 0·75 |
| Dextrin and sugar | .. | .. | .. | .. | .. | 3·79 |
| Starch | .. | .. | .. | .. | .. | 43·55 |
| Fat | .. | .. | .. | .. | .. | 0·70 |
| Ash | .. | .. | .. | .. | .. | 0·84 |

By these figures it is seen that there is a much larger proportion of solids, and also more soluble proteins and carbohydrates, in the crust than in the crumb. The crumb should be elastic in consistence, should have a sweet, nutty flavour, and be of a uniform whiteness throughout. As bread grows old, it becomes hard, and it has long been known that reheating softens it. The real explanation of the staling of bread does not seem to be known. Bibra holds that in fresh bread there is free water present, which, as staleness supervenes, unites with starch or gluten, and that reheating sets this water free. He states that the freshness will not return if the bread has lost 30 per cent. of its water. Others hold that the stale condition is produced by the shrinkage which takes place in the fibres forming the walls of the pores. The water vapour formed by the second heating drives these fibres apart again. It should be noted that during the baking of bread a large proportion of the fat is lost, amounting to as much as 7 per cent: in some instances, that the proteins are diminished from 1 to 2 per cent., and the carbohydrates from 3 to 4 per cent. Some of the starch is converted into soluble starch and dextrin to the extent of 8 per cent.

The estimation of **Water** and **Mineral Matter** in bread is performed as in the case of flour. Twenty grammes of the crumb make a convenient quantity with which to work.

The **Ash** is generally greater in weight than that of the flour used, owing to the sodium chloride, baking-powder, etc., added. Any excess of ash above 3 per cent. is generally regarded as due to salts added in order to improve the colour.

Silica is estimated by treating the ash with strong HCl and hot distilled water in a platinum dish, then filtering through a Swedish filter-paper, carefully washing the platinum dish with further boil-

ing distilled water, and transferring the washings to the filter-paper. When the residue on the filter has been several times washed with boiling distilled water so that all soluble substances have passed through, it is dried in a water oven, transferred to a porcelain crucible, ignited, and weighed as silica. It should not exceed 2 per cent.

Acidity.—Soak 5 grammes of bread in 50 c.c. of water for an hour. Filter and titrate the filtrate with $\frac{N}{10}$ NaOH, using phenolphthalein as indicator. The number of c.c. of decinormal soda used multiplied by 6 equals milligrammes of glacial acetic acid in 5 grammes of bread. The acidity should not exceed 0.11 per cent.

Adulteration.—Formerly **Alum** was used to whiten inferior flours, but at present it is practically never found. The detection of alum in bread is carried out as follows: Dissolve a small quantity of hæmatoxylin in alcohol, and to this add a little freshly prepared solution of ammonium carbonate in distilled water. Cubes of crumb are cut from the centre of a loaf, and small quantities of the solution poured upon them, after which they are removed to a water oven and dried at a low temperature. The production of a permanent lavender colour denotes the presence of alum. To a small degree magnesium salts simulate alum in this reaction, but the colour on drying is not so permanent. Silicate of alumina exists normally in flour, but in such small quantities that a 4-pound loaf will not contain more than 6 or 7 grains. The part played by alum when added to inferior flours is that of checking fermentation, which otherwise would lead to the production of glucose, and consequently a discoloured bread. It is stated that alum increases the porosity of bread. This adulterant has been found in quantities ranging from 20 grains to 100 grains per 4-pound loaf.

Quantitative Estimation.—Reduce $\frac{1}{4}$ pound of the bread to ash, and separate off the silica on a filter by treatment with strong HCl and boiling water in the usual manner. The filtrate contains phosphates of lime and magnesia, iron, and aluminium. To this solution add 5 c.c. of $(\text{NH}_4)\text{HO}$, which will precipitate all the phosphates, and 20 c.c. of strong acetic acid, which redissolves the phosphates of lime and magnesia. Filter and wash the residue of phosphates of iron and aluminium with boiling water. Dry,

ignite, and weigh. The residue is now dissolved in strong HCl, and diluted to 200 c.c., and the iron estimated colorimetrically. Convert the iron thus found into ferric phosphate by multiplying by 2.7, and subtract this from the weight of phosphates of iron and aluminium previously obtained; deduct also the weight of the filter ash, and the difference is aluminium phosphate, which may be returned as commercial alum (crystallized ammonium alum) by multiplying by 3.7.

Various cereal and casein preparations have appeared in recent years containing added **Phosphorus Compounds** in different forms—glycerophosphates, lecithins, and lipolins, etc. In order to measure these added substances it is necessary to estimate the phosphorus. This is easily effected by Neumann's method:

Prepare 2 litres of Neumann's molybdate-nitrate solution; dissolve 75 grammes of ammonium molybdate in 500 c.c. of water, and pour this into 500 c.c. HNO_3 ; add a litre of 50 per cent. ammonium nitrate solution.

Prepare mashed filter-paper for pressure filter. Place 30 grammes of minced filter-paper in a litre of water containing 50 c.c. HCl. Heat on water-bath with shaking for an hour. Filter. Wash with water repeatedly till all acid disappears. Leave in 2 litres of distilled water from which remove portions to pressure filter as required.

Decompose 0.5 gramme of the substance with nitric-sulphuric acid; add 60 to 100 c.c. of water and molybdate-nitrate solution in excess until solution remains clear on warming. Filter on pressure filter (about five minutes required). Add more molybdate-nitrate solution till all yellow precipitate is down. Wash the precipitate with water till free from acid (but not too long, as acid may separate out of the precipitate). Wash precipitate, pulp, and disc (used for supporting paper pulp in funnel) into a beaker; add about 300 c.c. of water and excess $\frac{N}{2}$ NaOH, and boil for ten minutes or so until NH_3 passes off. Titrate back with $\frac{N}{2}$ H_2SO_4 . Boil again to get rid of CO_2 , and finish the titration with a drop or two of $\frac{N}{2}$ NaOH.

One molecule $\text{P}_2\text{O}_5 = 56$ molecules NaOH.

1 c.c. $\frac{N}{2}$ NaOH = 1.268 milligrammes P_2O_5 .

[200 c.c. molybdate-nitrate solution = 0.1 gramme P_2O_5].

MEAT

The principal food animals are cattle, sheep, pigs, goats, horses, the buffalo and reindeer in a few countries, and in Saxony and Italy dogs.

Inspection of animals before slaughter is necessary for the detection of infectious diseases—anthrax, glanders, rabies, etc.—and for the discovery of intoxications in which meat and internal organs are but slightly altered.

Of the many pathological conditions which affect food animals these are of interest in laboratory work: Infective granulations; a few diseases produced by invisible organisms; and animal parasites.

Infective granulations are found, as tuberculosis, glanders, and actinomycosis.

To determine the extent of tuberculosis in slaughtered animals, it is necessary to make a methodical inspection of the hung-up carcase from above downwards. The meat is first examined, and afterwards the lymphatic glands, which receive the lymph from the meat, in the following order: (1) Popliteal, inguinal (superficial and deep), pubic, or supramammary lymph-glands. (2) Iliac and retro-peritoneal lymph-glands. (3) The lymph-glands along the sides of the vertebral column, ribs, and sternum. (4) Prescapular and axillary glands. (5) Pharyngeal and submaxillary lymph-glands. On completion of the examination of the lymphatic glands of the carcase, the internal organs with their lymphatic glands are next examined—viz., the kidneys and renal lymphatics, the spleen, liver, lungs, and the udder in female animals.

Lastly the peritoneum and pleuræ are systematically inspected. Actinomycosis occurs as small or large tumours delimited from the surrounding tissues by a thick wall of dense connective tissue in the jaws, tongue, skin of head and neck, and much more rarely in the lungs, liver, kidneys, udder, and abdominal wall.

Sections and smears containing *Bacillus tuberculosis* are readily prepared and stained with Ziehl-Neelsen's carbol-fuchsin, and counterstained with methylene blue.

Glanders must be distinguished from bovine farcy, not transmissible to man, and produced by a fungus of the genus *Discomyces*.

The ass is more susceptible to glanders than any other animal.

If a little discharge from the nose be rubbed into a few scarifications on the skin of the forehead, an œdematous swelling rapidly appears, followed by ulceration along the lines of the scratches; the temperature quickly rises to 40° C. or 41° C. The neighbouring glands swell, a discharge from the nose appears, and the animal dies in a few days. The chocolate-coloured growth of the glanders bacillus (*B. mallei*) on potato is characteristic. Microscopically *B. mallei* is a small straight rod (3 to 5 μ), with rounded ends. It is non-motile, non-sporing, and Gram-negative.

Sections and smears containing the filaments of actinomyces bovis stain well by Gram's method and by carbol-fuchsin. The microscopic appearances in both cases are unmistakable. Some difficulty may be experienced in isolating the parasite from pus in artificial culture, as the pyogenic organisms overrun the media before the actinomyces has had time to start. Spread pus containing the yellow granules on a couple of gelatin plates, and incubate at 22° C. for two days. Most of the grains will be surrounded by colonies of contaminating organisms, but a few will be found here and there discrete and isolated; pick these off with a stout platinum wire, and inoculate three or four coagulated serum slopes, and incubate at 37° C. In five or six days (note time as compared with a possible case of tubercle) colonies of actinomyces begin to grow. Sown in glycerin broth, hemispherical colonies appear in the same time (five to six days), as large as a small pea, and fall to the bottom, leaving the medium clear.

On glycerin-agar growth occurs in two days, which later becomes yellowish-white, dry, and wrinkled.

On potato in six to seven days small colourless colonies appear, which quickly become grey, yellow, and finally wrinkled and edged with black.

The *invisible organisms*, or so-called *filtrable viruses*, producing diseased conditions in food animals, are those of pleuro-pneumonia, foot-and-mouth disease, rinderpest, horse-sickness, swine-fever, cow-pox, sheep-pox, and bird-plague. Prior to 1898 laboratory methods failed utterly to throw any light on the causative agents operating in these diseases. In that year Nocard and Roux devised a new method of investigation.

In pleuro-pneumonia the essential lesion is the distension of the

meshes of the interlobar connective tissue with much clear, amber-coloured fluid. Subcutaneous inoculation of this fluid in another animal reproduces the disease, but the microscope and ordinary methods of cultivation are useless in searching for the micro-organism. Nocard and Roux filled collodion sacs sown with a drop of the fluid from a case of pleuro-pneumonia, and introduced them into the peritoneal cavities of rabbits. In two to three weeks the contents become cloudy. Microscopical examination with a magnification of 2,000 diameters show motile refractile points so small that their shape cannot be determined; these cannot be stained. By the twentieth day the virus has produced in the rabbit extreme emaciation, but no lesion; the organs and body fluids are sterile. The control animals in which similar but sterile sacs are inserted remain healthy. It would appear that rabbits are immune to the organism, but susceptible to the toxin. The contents of the sacs cannot be cultivated on ordinary media. After much experimentation these observers devised a medium on which the organism can be grown. This consists of 1 part of rabbit's serum and 20 parts of Martin's peptone solution (mix 200 grammes of cleaned and minced pigs' stomachs, 10 grammes of HCl, 1 litre of water at 50° C.; heat to boiling to destroy pepsin, and pass through cotton-wool; heat the filtrate to 80° C., and neutralize at this temperature; filter through Chardin paper, and autoclave the filtrate for four or five minutes at 120° C.; run 10 c.c. of the clear filtrate into test-tubes, and sterilize for twenty minutes at 115° C.). Tubes of this medium sown aerobically with a drop of exudate or of the contents of the collodion sacs, and incubated at 37° C., produce a virulent growth resembling in its microscopical and other characters that of the sacs.

This disease runs both an acute and chronic course. In the acute form the respiratory symptoms are most marked.

The exudate filtered through a Chamberland (F) bougie fails to produce the disease and to give cultures.

In experiments with ultramicroscopic viruses it is necessary to use a new and sterilized filter, and not to allow more than two hours for the filtration process, nor a temperature above 20° C. The pressure of filtration should be as low as possible, and the emulsion should be diluted to prevent blocking of the pores of the filter with

albuminous matter. Several animals should be inoculated with a large volume of the filtrate.

Foot-and-mouth disease infects cattle, sheep, goats, and pigs, and is transmissible to man. Aphthous lymph loses its infectivity when kept for five to six weeks. Löffler, by mixing such old lymph with fresh lymph, attenuated by heating for five minutes at 60° C., has been able to produce immunity in oxen.

Bird-plague virus has been grown by Marchoux on defibrinated fowl blood spread on glucose-peptone-agar, and incubated at 37° C. Growth occurs in the zone of blood adjoining the surface of the agar.

Animal Parasites in Meat.—Three groups of animal parasites may be recognized: I. Parasites not transmissible to man. II. Parasites which may be transmitted to man by eating meat. III. Parasites not immediately harmful, but which may become so after a preliminary change of host.

I. Parasites not transmissible to Man—1. *The Hair-Follicle Mite in the Skin of Hogs (Demodex phylloides suis)*.—It is from 0.2 to 0.25 millimetre long, and produces small swellings of the hair-follicles, greyish-yellow in colour, and containing disintegrated epithelial cells and dermal oil.

2. *Dipterous Larvæ*.—Larva of warble fly (*Æstrus bovis*), 28 × 15 millimetres, found in subcutis, causes considerable loss to cattle-raisers through deterioration of flesh and skins. It is found in the œsophagus from July to September, in the spinal canal from September to January, in the subcutis and skin from January to May. Other larvæ are the *Gastrophilus equi* and *G. nasalis*.

3. *Numerous Worms which appear in Organs of Food Animals*.—(a) All tapeworms except *Tænia echinococcus* of the dog, such as *Moniezia expansa* found in lambs, *Drepanido tænia lanceolata* and *D. setigera* in geese, *Davainea tetragona* in young fowls, *Tænia cænurus*, *T. marginata*, and *T. serrata*, in the dog. (b) Larval stages of all tapeworms, except *Cysticercus bovis*, *C. cellulosæ*, and *Echinococcus polymorphus*, such as *Tænia cænurus* (*Cænurus cerebralis*), which causes the disease known as 'gid' in sheep, and *Cysticercus tenuicollis* (larva of *T. marginata*) found in sheep, pigs, and cattle. (c) Flukes (Trematodes), such as *Distoma hepaticum* and *D. lanceolatum*, found in the liver of the sheep. These flat organisms measure as much as 25 × 13 millimetres. They are covered with scale-like

spines on the integument, which irritate the bile-ducts where they are located, and cause the thickening of these vessels so characteristic of the condition. They may wander from the liver to the lungs. Their embryonic stages are passed in a free condition in molluscs, mostly water-snails. Apart from the catarrh and cirrhotic condition of bile-ducts produced by these parasites, hæmorrhages occur, and the health of the affected animals may be seriously damaged. (d) Round-worms (Nematodes), with single exception of *Trichina spiralis*, such as *Ascarus*, *Eustrongylus*, *Filaria* (Schneider's group *Polymyaria*), *Oxyuris*, *Strongylus* (Schneider's *Meromyaria*), *Trichina spiralis*, *Trichocephalus*, *Anguillula* (Schneider's *Holomyaria*).

II. Parasites which may be transmitted to Man by eating Meat.—1. The Beef bladder worm (*Cysticercus bovis*), which is the larval form of *Tænia saginata* of man, known also as *T. medio-canellata*, consists of a somewhat elongated, roundish bladder located in the interfibrillar connective tissue of the striated musculature, and occasionally in lungs, liver, and brain. The grey transparent bladder consists of a connective-tissue capsule produced by reaction in surrounding tissues, and of the parasite. The latter consists of a scolex and caudal bladder filled with fluid; the scolex possesses four suckers, but no hooks. The size of the cysticercus varies from that of a pinhead to that of a small pea.

2. The Pork bladder worm (*Cysticercus cellulosæ*) is the larval stage of *Tænia solium*. In macroscopic appearances and location between muscle fibres it closely resembles *C. bovis*. For the rest, the cyst is more transparent, so that the scolex when invaginated into the caudal bladder appears more clearly. The scolex has twenty-two to twenty-eight hooks in a double circle; the hooks are of compressed shape, stout at the base and with slightly curved points. The cysticerci prefer the lumbar and abdominal muscles, pillars of the diaphragm, intercostal and masticatory muscles.

3. *Trichina Spiralis*.—Hilton investigated calcified trichinæ in 1832. Zenker discovered trichinosis in Dresden in 1860. After ingestion of trichinous meat, sexually mature trichinæ develop in the intestines of certain mammals; the parasite is set free from its capsule by the gastric juice. Males and females copulate, and the females deposit enormous numbers of embryos. Leuchart assumed that the embryos bore their way out of the intestine into the peri-

toneal and thoracic cavities, and ultimately reach the muscles. Heitzmann argues that this migration cannot possibly take place in the few days that elapse between the swallowing of infected meat and the appearance of embryos in the muscles, and that the embryos are conveyed by the blood-stream, and caught as emboli in the capillaries. Arrived in the muscles, a capsule is formed which in due course becomes calcified. The frequent occurrence of the parasite in rats is explained by the presence of the rat in abattoirs, knackers' yards, etc. Degeneration of trichinæ in their capsules frequently takes place. The muscles most likely to contain parasites are those of the tongue and larynx, and the pillars of the diaphragm.

Rhabditides (larvæ of strongylidæ) may be mistaken for trichinæ.

III. Parasites not immediately Harmful to Man, but which may become so after a Preliminary Change of Host—

1. *Echinococci*.—(a) *Tænia echinococcus* resides as a parasite in the small intestine of the dog, and is the asexual stage of a tapeworm with three to four segments. It is 2 to 6 millimetres long by 0.3 to 0.5 millimetre wide. It possesses a protruding rostellum with twenty-eight to fifty hooks. The last proglottid is 2 millimetres long, and contains mature eggs.

The echinococci occur in two chief forms—(a) *E. unilocularis* and (b) *E. multilocularis*. *E. unilocularis* forms simple cysts surrounded by connective tissue; in some cases daughter cysts are developed from the mother cysts, in other cases not.

E. multilocularis forms daughter cysts by constriction from a central mother cyst, which in turn are furnished with the same reproductive power. The daughter cysts do not remain in the mother cyst or inside the organic membrane formed about it, but after constriction become separated from the mother cyst by connective tissue. Accordingly, the vesicles attain no great size, but lie in the connective tissue like the epithelia of an acinous gland. The hooks of the multilocular form are somewhat larger than those of the unilocular.

The intermediate host is man.

The *E. unilocularis* occurs in the liver, lungs, and spleen, of the ox, sheep, and pig, and less often in the heart, kidneys, lymph-glands, muscles, and marrow cavities of bones.

The *E. multilocularis* occurs in the liver of bovines, forming tumours of various sizes which exhibit a constant growth.

2. *Larvæ of Pentastomum Tænioides*.—These are flat white structures, 4 to 5 millimetres long by 1 to 1.5 millimetres broad, divided into about eighty segments furnished with backwardly-directed tooth-like spines. Below the mouth there are two slit-like apertures on either side, from each of which the points of two claws protrude. These openings gave origin erroneously to the name *Pentastomum* (five-mouthed). The embryos are provided with a boring apparatus under the mouth opening, and at the opposite end of the body are several spines which serve for locomotion.

The parasites are found in hares, goats, sheep, and more rarely in cattle, under the peritoneum, in the liver, in the mesenteric glands, and in the lungs.

Dogs are the chief source of pentastome larvæ, and man, through intimate association with the dog, may become infected by ingestion of pentastome eggs.

A subdivision of the Protozoa—viz., the Sporozoa—are of some importance in meat inspection. This subdivision consists of the following orders: Coccidia, Myxosporidia, Sarcosporidia, and Hæmatosporidia.

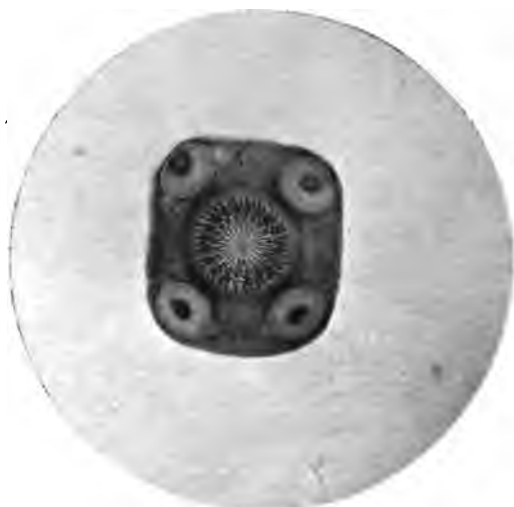
The Coccidia are parasites of epithelia, and occur in the liver of the rabbit and other animals, and occasionally in the liver of man. *C. perforans* occurs in the intestinal epithelium of rabbits, sheep, and calves, and causes a catarrhal diarrhœa.

Myxosporidia are chiefly parasitic in fish.

Sarcosporidia (Miescher's sacs) occur in hogs, mostly in the striated muscles.

Hæmatosporidia.—Theobald Smith's discovery of the organism of Texas fever in cattle conferred an importance on this group in relation to meat inspection, which with the constant discovery of new forms ever increases.

The flesh of different animals differs materially in appearance. Veal, mutton, and pork, are lighter in colour than beef. The method of slaughter has something to do with this, as in those cases where free bleeding takes place the flesh is of a lighter hue through loss of hæmoglobin. The flesh of young animals, containing as it does less hæmoglobin, is also lighter in tint.

FIG. 60.—HEAD OF CYSTICERCUS. $\times 20$.FIG. 61.—TÆNIA SOLIUM $\times 4$.

There is a widespread feeling in this country, not by any means founded upon knowledge, that the carcase of an animal which has



FIG. 62.—*TRICHINA SPIRALIS*. $\times 100$.



FIG. 63.—HEAD OF *DISTOMA HEPATICUM*. $\times 4$.

died of any disease should not be used as food. In certain cases this is obviously correct, but in others there is no evidence to show that the edible parts are in any way deteriorated as food materials.

Flesh containing infective parasites, and flesh which is in a state of putrefaction, including 'high' game, should be rigorously excluded from human consumption.

Good fresh meat possesses certain well-recognised characters which are easy of detection. It is firm and somewhat elastic to the touch, pointing to the fact that rigor mortis is well developed. It is dry on section, of a clear red colour and acid reaction. A section through the whole thickness of a joint presents a uniform appearance. The odour of fresh meat may be obtained by running a clean wooden



FIG. 64.—*ASCARUS LUMBRICOIDES*. $\times 7$.

skewer down to the bone, and withdrawing and smelling it. The fat is firm, and not too yellow in colour. Old animals and those fed on oil-cakes exhibit fat of a deep yellow colour. The bone-marrow is bright red, and coagulates within twenty-four hours.

The lymphatic glands are of normal size, colour, and consistence.

The ash contains a normal quantity of phosphoric acid and salts of potash.

When cooked, meat should not lose more than 30 per cent. of its weight, and when dried on a water-bath to constant weight it should not lose more than 75 per cent.

Bad meat may present many evil characters. A deep purple colour points to acute septicæmia, pulmonary disease ending in asphyxia, or when found in patches to hypostatic congestion. The odour may be that of advanced putrefaction, or it may be urinous, as in uræmia. There is absence of elasticity in a section when pitted with the finger; some parts are softer than others, and the flesh may be generally sodden and dropsical. The fat is highly coloured, soft, and perhaps hæmorrhagic. The juice expressed from unsound meat is alkaline in reaction from the formation of



FIG. 65.—*OXYURIS VERMICULARIS*. $\times 20$.

ammonias. At later stages the meat becomes green, and even black, when no critical examination is required to establish its condition. Certain chemical tests have been devised to detect putrefaction in the early stages, but none of them can convey more reliable information than that obtained by well-trained eyes and noses.

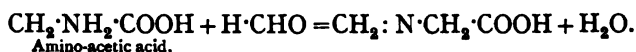
The carcasses of animals that have died of anthrax and allied conditions, pyæmia, and septicæmia, present congested, ecchymosed, and hæmorrhagic tissues. In all cases where one or other of these diseases is suspected the offal should be seen and carefully examined.

Preservatives in Meat—Boric Acid.—A portion of finely-divided meat mechanically freed from fat is warmed with water acidulated with HCl. The extract is tested with turmeric. Quantitative estimation is made from the same extract as under milk.

Salicylic Acid.—A portion of meat freed from fat as above is slightly acidified and shaken up with ether; the ether extract is evaporated to dryness, and the residue tested in aqueous solution with ferric chloride. A violet colour indicates salicylic acid.

Formaldehyde.—This preservative may be used as a solution, and as a gas in meat-safes and the holds of vessels carrying chilled meats. Inside safes is placed a receptacle carrying pastilles of polymerized formaldehyde—paraformaldehyde or trioxymethylene—which is heated until the paraformaldehyde is depolymerized and simple aldehyde vapour is given off. The meat is left in contact with the vapour for twenty minutes or more. In the holds of vessels formalin is evaporated in the presence of the quarters of dressed meat in the proportion of 10 ounces to 1,000 cubic feet of space. Formaldehyde penetrates the substance of the meat, especially areas not covered by fat, to distances extending from 5 to 20 millimetres.

The proteins and amino-acids of meat unite with formaldehyde to form methylene-imino compounds, as demonstrated by Schiff. The reaction is reversible, and only proceeds to completion in the presence of excess of formaldehyde:



Amino-acids composed of basic and acid groups have an amphoteric reaction; when treated with H·CHO they become acid, and the amount of liberated acid can be readily determined by titration with standard alkali: hence the amount of formaldehyde which enters into the reaction can be determined.

The colour reactions by which formaldehyde can be detected in milk are not applicable to meat, inasmuch as meat gives a violet colour when heated with HCl in the absence of formaldehyde (formation of hæmatoporphyrin from Hb).

Schryver uses the following test: To 10 c.c. of the water in which a portion of meat has been heated for five minutes in a boiling water-bath, add 2 c.c. of a 1 per cent. phenylhydrazine hydrochloride

solution. Cool and filter through cotton-wool. Add 1 c.c. of 5 per cent. potassium ferricyanide solution and 4 c.c. of concentrated HCl. A brilliant fuchsin-like colour is formed, which in a few minutes reaches its maximum and lasts for several hours. (The ferricyanide oxidizes the aldehyde condensation product to a body which is a weak base, which forms a scarlet hydrochloride. On dilution with water this body hydrolyses, forming a base which can be extracted with ether to form a yellow solution. If to this last concentrated HCl be added, the base passes back into aqueous solution in the form of the scarlet hydrochloride.)

In those cases in which the formaldehyde amounts to about 1 in 50,000 parts of meat, 10 grammes of minced meat are used with 10 c.c. of water. Where the concentration reaches 1 part in 5,000 meat, 10 grammes of meat are heated with 100 c.c. of water and 20 c.c. of the phenylhydrazine hydrochloride solution. After filtering and cooling, 12 c.c. of the filtrate (as above) are mixed with 1 c.c. of the ferricyanide and 4 c.c. HCl.

By comparing the colour obtained with carefully prepared standards, the amount of formaldehyde in any sample of meat can be determined (*see* Appendix).

Bacterial Food-Poisoning (*cf.* L.G.B. Food Reports, No. 18).—Three groups of bacteria appear to take part in outbreaks of food-poisoning—viz., the Gärtner group of bacilli; *Bacillus coli*, *B. proteus*, etc.; and *B. botulinus*.

The Gärtner group (*B. enteritidis*, *B. suispestifer*, *B. paratyphosus B*, etc.) has been found responsible for many outbreaks of poisoning through eating pork, pork pies, pork sausages, brawn, meat and minced and baked meat, tinned tongue, tinned salmon, veal pies, milk, etc.

The *B. coli* group has been found in milk, meat pies, tinned meat, etc. *B. proteus* and other putrefactive bacteria are occasionally found in cases of poisoning by sausages, chilled meat, etc.

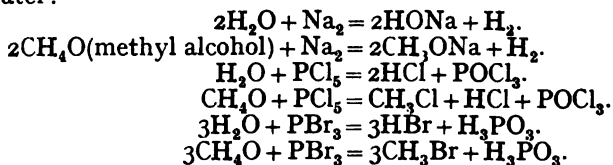
B. botulinus (studied by Van Ermengem) is occasionally responsible for cases of sausage-poisoning.

An experimental investigation in the human subject on the influence of boric acid and borax on food, by Dr. Harvey W. Wiley, United States Department of Agriculture, was published in 1904, as Bulletin No. 84, part 1, Bureau of Chemistry, Washington; and

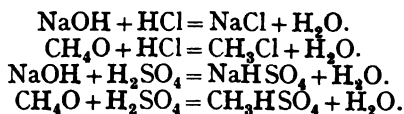
a further similar investigation on the influence of salicylic acid and salicylates was published in 1906, as part 2 of the same bulletin. Wiley's findings on the influence of boric acid and borax were critically reviewed by Professor Oscar Liebreich; an English translation of Liebreich's report, dated 1906, is published by J. and A. Churchill.

ALCOHOLIC BEVERAGES

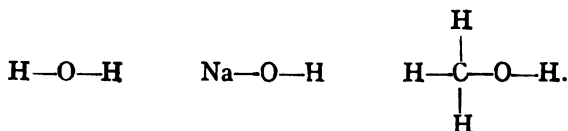
The alcohols ($C_nH_{2n+2}O$) may be regarded as oxygen derivatives of the paraffins. They are colourless and neutral substances possessing neither alkaline nor acid reaction. Those with few carbon atoms are liquid; the higher members of the series are solid. Methyl, ethyl, and propyl alcohols are miscible with water; butyl alcohol dissolves in 12 parts, amyl alcohol from fusel-oil requires 39 parts of water. The relative proportion of oxygen determines the solubility in water; as oxygen decreases with increasing molecular weight, the physical characters of the paraffin correspondingly predominate. Alcohols resemble water in certain reactions, in others caustic alkalies. They, like water, liberate one atom of H when treated with sodium, and retain as a substitute one atom of the latter. The action of P, Br, etc., on alcohols results in compounds similar in structure to those formed from water:



The similarity in constitution between alcohols and caustic alkalies is seen by the following reactions:



It follows, then, that the graphic formula of an alcohol may be constructed in the same manner as that for water and caustic soda:



The different alcohols do not behave alike on oxidation. Some form aldehydes, others ketones. This difference in behaviour on oxidation divides them into three groups—primary, secondary, and tertiary alcohols. A primary alcohol has the hydroxyl group linked to an end carbon atom of a straight chain, and contains the group $\cdot\text{CH}_2(\text{OH})$. A secondary alcohol has the hydroxyl group attached to a middle carbon atom of a straight chain, and contains the group $:\text{CH}(\text{OH})$. In a tertiary alcohol the carbon atom attached to the hydroxyl group is linked to three carbon atoms $:\text{C}(\text{OH})$.

Methyl alcohol, $\text{CH}_3(\text{OH})$, has a specific gravity 0.812, and boiling-point 66°C .

Ethyl alcohol, $\text{C}_2\text{H}_5(\text{OH})$, has a specific gravity 0.806, and boiling-point 78°C .

Propyl alcohols, $\text{C}_3\text{H}_7(\text{OH})$.

Propyl alcohol (primary), $\text{CH}_3\text{CH}_2\text{CH}_2(\text{OH})$, has a specific gravity 0.804, and boiling-point 97°C .

Propyl alcohol (secondary), $\text{CH}_3\text{CH}(\text{OH})\text{CH}_3$, has a specific gravity 0.789, and boiling-point 81°C .

Butyl alcohols, $\text{C}_4\text{H}_9(\text{OH})$.

Butyl alcohol (normal primary), $\text{C}_2\text{H}_5\text{CH}_2\text{CH}_2\text{OH}$, has a specific gravity 0.810, and boiling-point 117°C .

Butyl alcohol (normal secondary) $\text{C}_2\text{H}_5\text{CH}(\text{OH})\text{CH}_3$, has a boiling-point 100°C .

Butyl alcohol (tertiary), $(\text{CH}_3)_3\text{C}(\text{OH})\text{CH}_3$, has a specific gravity 0.786, and boiling-point 83°C .

Amyl alcohols, $\text{C}_5\text{H}_{11}(\text{OH})$.

Normal primary, $\text{C}_2\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2(\text{OH})$, has a specific gravity 0.815, and boiling-point 138°C .

Isobutyl carbinol, $(\text{CH}_3)_2\text{CH}\text{CH}_2\text{CH}_2(\text{OH})$, has a specific gravity 0.810, and boiling-point 131°C .

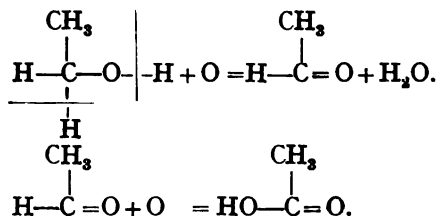
Secondary butyl carbinol, $\text{CH}_3\text{CH}(\text{C}_2\text{H}_5)\text{CH}_2(\text{OH})$, has a boiling-point 128°C .

Methyl propyl carbinol, $\text{C}_2\text{H}_5\text{CH}_2\text{CHOH}\text{CH}_3$, has a boiling-point 119°C .

Diethyl carbinol, $\text{C}_2\text{H}_5\text{CHOH}\text{C}_2\text{H}_5$, has a boiling-point 117°C .

The primary alcohols on oxidation lose two atoms of hydrogen and form aldehydes; the latter, on continued oxidation, take up one atom of oxygen, and are converted into acids.

Ethyl alcohol yields acetaldehyde, and then acetic acid:



The secondary alcohols yield up two atoms of hydrogen in the first stage to form ketones. Further oxidation forms acids containing fewer carbon atoms than the ketones.

The tertiary alcohols decompose on oxidation, forming ketones, or acids containing fewer carbon atoms than the alcohol. The alcohols are found as constituents of many natural products, such as fats, oils, waxes, etc. They are prepared mainly by fermentation. Ethyl, propyl, butyl, and amyl alcohols are all produced in this way. Methyl alcohol is obtained by the distillation of wood, and by the destructive distillation of the by-products of the beet-sugar industry. Commercial methyl alcohol contains acetone.

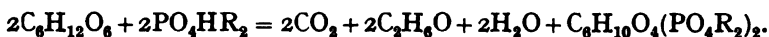
When yeast is added to a solution of grape-sugar or cane-sugar, the liquid froths and appears to boil; the sugar is broken up into ethyl alcohol and carbon dioxide. Pasteur described this as the result of life without oxygen, the yeast cells being able in the absence of free oxygen to use combined oxygen liberated in the decomposition of the sugar or other substance. Many explanations of the phenomenon were offered by observers in a controversy which has lasted for many years.

In 1896 Buchner discovered accidentally that yeast-juice (free from cells), to which sugar had been added in order to prevent putrefaction, fermented the sugar; on heating the juice to 50° C. its power of fermentation was destroyed. He concluded that the production of alcoholic fermentation does not require so complicated an apparatus as the yeast cell, and that fermentation was effected by a dissolved substance in the cell to which he gave the

name of "zymase." Yeast-juice contains a powerful tryptic enzyme. Zymase when it has acted for some time disappears, and Buchner concluded that it was destroyed by the endotrypsin. When a mixture of alcohol and ether is added to juice, a precipitate is formed which can be dried to an amorphous powder (zymin) of high fermentative activity.

The action of living yeast appears to follow the same law as that of most enzymes—viz., the enzyme unites with the fermentable material (substrate or zymolyte), forming a compound which only slowly decomposes, so that it remains in existence for a perceptible interval of time. The rate of fermentation depends on the rate of decomposition of this compound, and hence varies with its concentration.

It has been shown by Harden that the addition of a soluble phosphate to a fermenting mixture of a hexose with yeast-juice or zymin causes the production of an equivalent quantity of carbon dioxide and alcohol, which fact, it is concluded, indicates that a definite chemical reaction occurs in which sugar and phosphate are concerned. An equation can be constructed embodying two molecules of sugar in action in which carbon dioxide and alcohol are equal in weight to half the sugar used, and hexosephosphate and water to the other half:



The main difference between fermentation by yeast-juice and by the living cell appears to consist in the rate of decomposition of the hexosephosphate. A comparison of living yeast, zymin, and yeast-juice, shows that these form an ascending series with respect to their response to phosphate. Using fructose as the zymolyte, yeast does not respond to phosphate at all, the rate of fermentation by zymin is doubled, and that by yeast-juice increased twenty to forty times. It may be that the balance of enzymes in the living cell is such that the supply of phosphate is maintained at the optimum, and a further supply, consequently, does not alter the rate of fermentation.

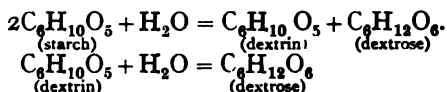
Although alcohol is the principal constituent by which such beverages affect the nutrition of the body, it must not be forgotten that in many cases ethers, aldehydes, and other by-products of

fermentation, are likewise found. Alcohol to the extent of 1 per cent. seems to be favourable to a digesting mixture in the stomach; 10 per cent. slightly retards gastric digestion, and 20 per cent. arrests it. Pancreatic digestion is much more sensitive to alcohol; but as digestion is not only a chemical process, but greatly influenced by the movements of the stomach and other factors differing widely in different individuals, it is not surprising to find that alcohol has very different effects in its relation to individual cases. It is admitted on all hands that it quickens the activity of stomach movements and secretions. If the retarding influence of alcohol on the chemical part of digestion be weighed against its quickening influence on the flow of gastric juice and on gastric peristalsis, the balance is in favour of its use as a digestive stimulant. In certain conditions of disease these properties are greatly enhanced. Alcohol, unlike water, is freely absorbed by the mucous membrane of the stomach, and requires no digestion. It passes into the blood at once. Not only is it rapidly absorbed itself, but it assists the absorption of other bodies. Whilst it passes from the stomach into the blood, water passes from the blood into the stomach; the endosmotic equivalent of alcohol is 4.2, which means that, for every gramme of alcohol passing through an animal membrane in one direction, 4.2 grammes of water pass in the opposite.

Alcoholic beverages are all in a broad sense saccharine products, the result of the fermentation of sugar. In fruits sugar exists in the juice, which on exposure to the air ferments:



In grain a preliminary fermentation takes place—starch is converted into sugar:



Beer.

In making beer, barley is steeped in water and spread in layers a few inches deep on floors, where a temperature favourable to germination is maintained. Diastase is formed in the grain. When germination has proceeded sufficiently, the grain is dried on a kiln,

and is known as malt. The malt is mixed with water at 60° to 65° C., and the diastase rapidly converts the starch into dextrin and maltose. The extract, or wort, is run into copper pans and boiled, with addition of hops. The liquid is now rapidly cooled to 15° to 17° C. and drawn into vats; yeast is added, and the maltose alone undergoes fermentation. As this sugar forms only a small portion of the extract, the quantity of alcohol is not large. The addition of glucose to the boiling-pan increases the amount of alcohol.

The wort is capable of growing other bacteria than yeast, and if great care is not taken secondary fermentations occur, and produce diseased beers.

In brewing, the temperature largely affects the character of fermentation. Slow fermentation, known as 'bottom fermentation,' in which the yeast settles out at the bottom, proceeds at 6° to 8° C. Top fermentation, in which the yeast is carried to the surface, occurs at 16° to 18° C., and is not so easily controlled. The yeast cells in either case feed on the dextrin, maltose, peptones, and amides of the wort.

Lager beers contain a low proportion of hops (female flower of *Humulus lupulus*) and a high proportion of extract and alcohol.

At the proper phase beer is drawn off the yeast and run into casks, where it undergoes a secondary fermentation.

Most of the German white beers are produced by quick top fermentation, and have a high percentage of carbon dioxide, being bottled before the second fermentation is complete.

English ale is made by top fermentation of a wort which contains a considerable proportion of hops. The fermentation is checked at an early stage, hence it is rich in sugar.

Porter is a dark ale made from brown malt dried at a high temperature. It has a large extract, mainly sugar, and may contain caramel.

Stout is porter with larger alcohol and extract contents.

Detection of Ethyl Alcohol.—Warm 10 c.c. of the fluid under test with a few drops of benzoyl chloride; add a little NaOH solution; ethyl benzoate is formed with characteristic odour where as little as 0.1 per cent. alcohol is present. Other alcohols produce ethers with characteristic odours.

The Iodoform Test.—Warm 10 c.c. of the fluid in a test-tube

with a few drops of strong solution of iodine in KI; add solution of NaOH till the mixture is nearly decolourized. On standing a precipitate of iodoform (star-shaped or hexagonal tablet crystals) forms where alcohol is present to the extent of 0.1 per cent.

Acetone, lactic acid, and certain aldehydes and ketones, give this reaction, but not pure methyl alcohol, amyl alcohol, or acetic acid.

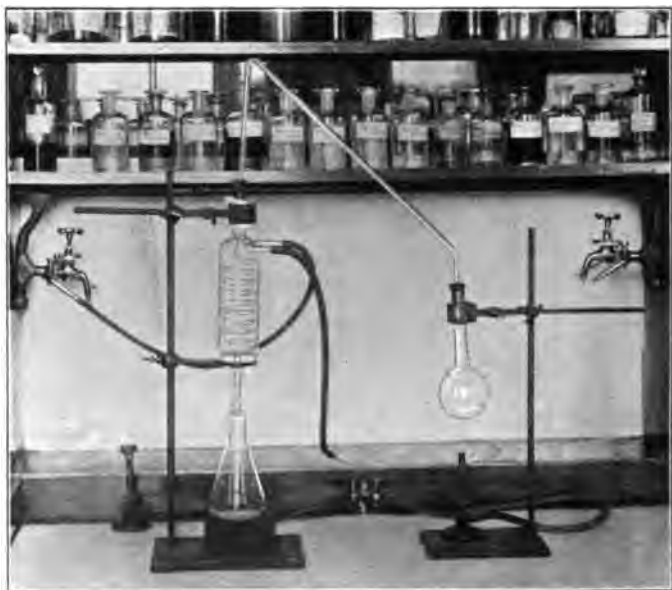


FIG. 66.—ESTIMATION OF ALCOHOL.

Estimation of Alcohol.—Expel free CO_2 by shaking in a flask or separator funnel and drawing the still liquid away from the froth.

Into a 250 to 400 c.c. flask pour 100 c.c. beer; add some tannic acid to prevent frothing; dilute to about 150 c.c. with H_2O and distil. All the alcohol will come over in the first 75 c.c. distillate—*i.e.*, three-fourths the original measured volume. In the case of liquors high in alcohol, it is better to distil over about 100 c.c. Make up the distillate to the volume of the liquor originally taken

and shake well. Take the specific gravity in a pycnometer. Refer to the alcohol table, and read off the percentage of alcohol by volume or by weight.

Tabarie's Method.—Find the specific gravity of the beer. Evaporate 100 c.c. on a water-bath to one-fourth the volume. Make up to the original volume with distilled water, and find the specific gravity of the dealcoholized fluid. Add 1 to the original specific gravity, and from the sum subtract the second specific gravity. The difference is the specific gravity corresponding to the alcohol in the liquor. Suppose the specific gravity of the sample to be 1.9899, and that of the dealcoholized sample 1.0099. Then $1.9899 - 1.0099 = 0.9800 = 16.24$ per cent. alcohol by volume.

Acidity.—The total acidity is usually expressed in terms of lactic acid. Measure 20 c.c. beer and free it from CO_2 by raising it to the boiling-point. Cool, and titrate with $\frac{N}{10}$ NaOH, using litmus as indicator. 1 c.c. $\frac{N}{10}$ NaOH = 0.009 gramme lactic acid.

The Fixed Acid expressed as Lactic.—Evaporate 20 c.c. beer to one-fourth its volume, dilute with water to original volume; titrate with $\frac{N}{10}$ NaOH as before.

Volatile Acid expressed as Acetic.—Distil 100 c.c. beer nearly to dryness. Should the residue in the retort be still acid, add some water and continue the distillation to dryness. Now titrate the distillate with $\frac{N}{10}$ NaOH, each cubic centimetre of which = 0.006 gramme acetic acid. The normal acidity of beer is due to CO_2 , acetic, lactic, malic, and other organic acids, and should not exceed in 100 c.c. that neutralized by 30 c.c. $\frac{N}{10}$ NaOH.

The Malt Extract.—To estimate this item with any degree of accuracy, a small quantity must be operated on. Take 5 c.c. or 5 grammes in a large platinum dish so that a thin film is formed on the bottom. Dry for two or three hours on the water-bath, and finish the drying in an air-bath at a temperature somewhat above 100°C .

Bitters.—The bitter of hops is readily soluble in ether; the bitters of quassia, aloes, and hop substitutes, are insoluble in ether; whilst many bitters that might be employed are soluble in ether, the absence of a bitter taste from the ether extract demonstrates the absence of hops. In performing the test, evaporate the beer to the consistence of a syrup before extracting with ether. Further, lead

acetate completely precipitates the bitter material of hops, but leaves behind some of the bitters of hop substitutes, which may be recognized on concentrating the filtrate.

Aloes.—Dry 200 c.c. beer and treat the residue with ammonia. Filter, cool, and treat the filtrate with HCl. Collect the resin on a filter. This is insoluble in cold water, ether, petroleum ether, and chloroform, but soluble in alcohol. It has a characteristic odour which identifies it.

Gentian.—Treat the acid residue with chloroform in the cold: no colour is produced; warm, and a carmine-red colour appears. A small quantity of the red solution mixed with a drop or two of ferric chloride solution changes to a greenish-brown.

Quassia.—Quassiin in acid solution is soluble in chloroform, and, when mixed with a little alcoholic solution of ferric chloride, gives a mahogany-brown coloration.

Preservatives in Beer.—*Boric acid and salicylic acid* are detected in the same manner as described under milk, concentrating the beer if necessary to one-fifth or one-tenth of the bulk. Saccharin is detected by acidulating a portion with H_2SO_4 , shaking with a mixture of equal volumes of ether and petroleum spirit, evaporating down with a little NaOH solution, and carefully heating for a short time to about 250°C . Salicylic acid is formed, and this is tested for in the ordinary way.

Sulphurous Acid.—To 25 grammes sample in a 200 c.c. flask add 25 c.c. N-KOH. Shake and set aside for twenty minutes. Add 10 c.c. 25 per cent. H_2SO_4 and a little boiled starch solution. Titrate rapidly with $\frac{\text{N}}{10}$ iodine till a blue colour is produced. One c.c. of the iodine solution = 0.00064 gramme SO_2 . Sulphurous acid is used to regulate the fermentation and to produce a flavour of age.

Sodium Chloride.—Where common salt has been added, an allowance not exceeding 50 grains per gallon must be made for the amount of this compound present in the water, malt, and hops used. Ash a suitable quantity of beer, say 100 c.c.; exhaust the ash with water; titrate the solution with $\frac{\text{N}}{10}$ AgNO_3 , using neutral potassium chromate as indicator.

Arsenic.—In Lancashire in 1900 an outbreak of arsenical poisoning occurred, in which arsenic amounting to $\frac{1}{10}$ grain per gallon was fre-

quently found, and it was stated in some cases that 1 grain in a gallon was found.

Marsh Test—Preliminary Treatment of Beer.—Place 100 c.c. beer freed from CO_2 by shaking in a porcelain dish; add 20 c.c. pure concentrated HNO_3 and 3 c.c. concentrated H_2SO_4 ; heat in a fume chamber till vigorous frothing occurs; lower the flame and stir till frothing ceases; boil freely; continue heating till mass chars and fumes of H_2SO_4 are given off; pieces of filter-paper may be stirred in till the residue is dry; cool, add 50 c.c. water, and remove masses of char from sides of dish with glass rod; heat to boiling and filter; use the filtrate in the Marsh apparatus.

Marsh Apparatus.—Fit up a generating flask with funnel tube. Attach a U-tube containing pumice moistened with 10 per cent. lead acetate solution to absorb H_2S . To this attach a CaCl_2 drying tube, and a hard glass tube of about 6 millimetres bore drawn out for about 4 centimetres to 1 millimetre internal diameter; draw out the end to still narrower dimensions; support the tube over a two- or three-burner furnace, wrapping the portion in contact with the flame in wire gauze.

Place in the generating flask 20 to 30 grammes arsenic-free stick zinc and a perforated platinum disc to form an electric couple. Run in through the funnel sufficient 20 per cent. H_2SO_4 to start the reaction and expel air. When all air has been driven out and danger of explosion has passed, heat the tube to bright redness. When absence of As in the reagents has been settled, add slowly through the funnel the solution of the substance in 20 per cent. H_2SO_4 . When the flow of gas begins to slacken, add some 30 per cent. H_2SO_4 , and later 40 per cent. acid, till all As has been expelled. Two or three hours may be required to finish the expulsion. If no mirror forms in the constriction of the tube in an hour, it may be taken that there is no As present.

If more than 0.1 milligramme As appears to be present, cut off the constriction from the tube and weigh it on a fine balance. Dissolve the As out with a solution of sodium hypochlorite; wash the tube with water; dry with alcohol and weigh. The loss of weight is As.

If the As is very small in amount, compare the mirror with a series of standard mirrors prepared in the same apparatus from

quantities of a standard solution of As containing from 0.005 to 0.05 milligramme As_2O_3 . Such standard solution is prepared by dissolving 0.1 gramme pure As_2O_3 in a little pure NaOH solution, acidifying with pure H_2SO_4 , and making up to 100 c.c. with water. Ten c.c. of the latter fluid is further made up to 1 litre. One c.c. = 0.01 milligramme As_2O_3 .

Reinsch's Test.—Acidify 100 c.c. beer with 1 c.c. HCl (free from arsenic); evaporate to less than half the bulk. Set up two beakers on gauze over Bunsen burners (the second to act as a control). In the first place the prepared beer, and in the second an equal volume of water. To each add 5 c.c. concentrated pure HCl and a strip of bright pure copper-foil 10 millimetres by 5 millimetres. Heat for an hour, replacing from time to time the water lost by evaporation. If a deposit forms on the copper, remove it, and wash very carefully with water, alcohol, and ether. Place in a subliming tube and heat over a low flame. The crystals are for the most part regular octahedra, with perhaps a mixture of rectangular prisms.

Clarke has made this test quantitative: Dissolve the arsenic from the Cu slip in dilute aqueous solution of potash and H_2O_2 in the cold. Then boil, and filter off any CuO. Concentrate the filtrate to a small bulk and wash into a distilling flask with strong arsenic-free HCl; add some ferrous chloride; fit the flask with a safety tube and connect with a small worm condenser. Distil down twice with pure strong HCl. Pass H_2S into the distillate. A precipitate will form if more than 0.1 milligramme be present; if less than this quantity, a yellow colour. As little as 0.001 milligramme arsenic sulphide gives a faint yellow colour, which may be matched by a series of standard colours produced under the same conditions.*

Wine.

In making wine the juice of the grape is left in open vats where its sugar undergoes spontaneous fermentation. The bloom which covers the outside of the grape contains the necessary yeast, and the natural acidity of the juice, or must, excludes foreign organisms.

* See description of Marsh-Berzelius process, *Analyst*, February, 1902, xxvii., 48, 210.

The relative proportions of protein and sugar influence the character of the wine, as yeast (*Saccharomyces ellipsoideus*) lives upon the protein, and splits the sugar, forming alcohol and other products. If yeast grow in little sugar and much protein, it can maintain its existence until all the sugar is changed; such a wine is said to be dry and acid, like hock. Conversely, if there be much sugar and little protein, the growth of yeast comes to an end before all the sugar is used, and that left behind produces a sweet wine. Intermediate proportions of sugar and proteins produce corresponding results. It may be noted, though, that, no matter what the proportions of protein and sugar, fermentation cannot proceed after 16 volumes per cent. of alcohol have appeared in the liquid; this is why a natural wine can never contain more than this proportion of alcohol. Sherry and port are fortified wines—that is, containing, as they do, more than 16 per cent. of alcohol, they have the difference added to them. Claret and hock are natural wines. The quality of wine depends on the species of yeast used, the variety of grape, the soil and climatic conditions of growth of the grape, and the mode of its cultivation. The colour of red wines is produced by a pigment (*xenocyanin*) residing in the skins of the grapes, which is turned red by the acids present. As alcohol is produced, it dissolves out this pigment, and so colours the distillate. Wine, when placed in casks, undergoes important changes: water evaporates more quickly through the woodwork than does the alcohol, and so the alcohol becomes concentrated. Further, some oxidation of the tannic acid takes place; this causes white wines to be somewhat darker in colour, and red wines lighter, through the carrying down of some of their pigments by oxidized tannic acid. Frequently a small amount of yeast enters the cask, and continues the fermentation, thereby increasing the quantity of alcohol. With the lapse of time, some of the alcohol is oxidized into acetic acid, and certain compound ethers are formed. Wine in bottles adds to its contained ethers, although its alcoholic strength rarely, if ever, increases. It is an error to suppose that very old wine contains most alcohol: slow oxidation in the case of wines, as in all other organic compounds, produces degeneration. It is more than probable that no wine improves in quality after a period of ten to fifteen years.

Fermentation progresses most rapidly at a temperature between 25° and 30° C., but finer bouquet is produced by slower fermentation, and accordingly must be fermented in open vats in cool cellars at 5° to 15° C. till it settles out comparatively clear, care being taken to avoid acetic fermentation. When the first or active fermentation is complete, the wine is drawn off into casks, where it undergoes a second slow fermentation, with deposit of potassium bitartrate and development of the characteristic flavour. The wine is sometimes clarified with gelatin, and sometimes pasteurized, before the final bottling or casking. Volatile ethers predominate in natural wines, fixed ethers in fortified. Sparkling wines, as distinguished from still, are highly charged with CO₂, either produced naturally by after-fermentation of added sugar (champagne), or artificially by carbonating, as in the case of soda-water.

Port wine is rich in tannin, and to certain inferior wines this astringent, together with alum and catechu, is added. Port contains a large amount of extracts, which give it a full body, and old port a large proportion of ethers, of which (unlike sherry) the fixed ethers predominate over the volatile.

Sherries, as imported into this country, are all fortified and plastered, and contain from 15 to 25 per cent. of alcohol by weight. Old sherry contains a large proportion of volatile ethers, and to this property much of its value as a stimulant must be attributed.

Champagne is produced from black grapes, and depends for its character very largely upon the quality of the grapes of a particular vintage. The expressed juice, after sedimentation for twelve hours, is drawn off and fermented; it is then bottled and allowed to undergo secondary fermentation for a couple of years, during which time much CO₂ is produced, and a deposit. To the wine, which is up till now sour, cane-sugar, which has been dissolved in old champagne, is added in varying quantities. Dry champagnes which find their way to England contain little sugar—not more than 1 or 2 per cent., whilst sweet champagnes may contain 10 to 15 per cent.

Claret is a deep red wine, somewhat acid and astringent; it contains little sugar, but considerable quantities of volatile ethers. Its content of alcohol varies from 8 to 12 per cent. by volume.

Hock is a white wine containing little sugar, 9 to 12 per cent. by volume alcohol, and is mildly acid.

Plastering is the term applied to the adulteration of the must before fermentation with plaster of Paris or gypsum, wherein objectionable potassium sulphate is left in solution in the wine:



The precipitation of calcium tartrate carries down impurities, the colour is improved, and the fermentation hastened and made more complete; the practice is said to enhance the keeping qualities of the wine.

Cane-sugar is added to the must to increase the yield of alcohol. Glucose is used instead of cane-sugar, and introduces unfermentable matter, dextrin, and various mineral salts.

Added Water.—Gautier ('*Traité sur la Sophistication et l'Analyse des Vins*') has shown that the sum of the weight in grammes of alcohol in 100 c.c. and total acidity (as H_2SO_4) in a litre varies in pure wines within narrow limits, being rarely below 13 or above 17. If considerably below 13, water may be assumed to have been added.

Colouring Matter in Wine.—Cubes of solid transparent gelatin, $\frac{1}{4}$ inch square, are immersed in the wine for twenty-four hours, after which they are removed, washed in water, and cut in half. In genuine wines the colouring matter will not have penetrated more than one-sixteenth of an inch, whilst in wines coloured with fuchsin, cochineal, logwood, litmus, indigo, etc., the cubes will be penetrated to the centre. The colouring matter of alkanet root, turned blue by ammonia, is the only foreign matter in general use which slowly penetrates the gelatin. Dilute ammonia dissolves cochineal and logwood out of the gelatin, the cochineal becoming purple and the logwood brown.

Estimation of Alcohol.—As in beer.

Acids.—The acids of wine are chiefly tartaric, malic, and tannic, and certain acids of the fatty series—acetic, formic, etc.—produced during fermentation. Tartaric acid forms with potassium a bitartrate. As alcohol increases in wine this salt becomes less soluble, and finally falls out in the form of a crust, so that the acidity diminishes on keeping. Tannic acid is obtained from the skins and stalks of the grapes used; it diminishes by oxidation on keeping, and in old wines is small in amount.

Get rid of CO_2 by shaking. Heat about 20 c.c. to boiling, and titrate with $\frac{N}{10}$ NaOH (in white wines and cider use phenolphthalein as indicator). One c.c. $\frac{N}{10}$ NaOH = 0.0067 gramme malic acid, or 0.0075 gramme tartaric acid. This is the total acidity.

Volatile Acids.—Place 50 c.c. with a little tannin in a distilling flask connected with a condenser. Connect a second distilling flask containing 250 c.c. water with the first by glass tube passing almost to the bottom. Heat both to boiling; then lower the flame under the distilling flask and pass steam through the wine until 200 c.c. distillate come over. Titrate the distillate with $\frac{N}{10}$ NaOH (indicator phenolphthalein). One c.c. $\frac{N}{10}$ NaOH = 0.006 gramme acetic acid.

Ethers.—Ethers are produced in wines by the chemical action which takes place between the acids and alcohols. Volatile ethers are obtained from volatile acids, such as acetic, and these, especially acetic ether, predominate in natural wines. Fixed ethers are derived from fixed acids, such as tartaric, and are found in fortified wines; they impart to wine its bouquet. Ceanthic ether 1 part in 50,000 wine imparts the vinous smell and taste to all wines in common.

Extract.—Dry 10 grammes to constant weight in a platinum dish; a small amount of glycerin may be lost.

Ash.—Ignite the dried residue at a low temperature and weigh. Most natural wines contain 1 part ash to 10 parts extract.

Sugars.—The chief sugar of wine is lævulose, of which a natural wine should not contain more than 0.5 per cent. Fortified wines may contain from 2 to 25 per cent. Extractives found in wine consist of gums and various carbohydrates, and contribute to the taste and so-called body of the wine. Reducing sugars are determined by Fehling's method.

Potassium Sulphate.—Acidify 100 c.c. of the wine with HCl; boil and add excess BaCl_2 . Filter, wash well, dry, ignite, weigh as BaSO_4 ; calculate the equivalent K_2SO_4 . More than 0.06 gramme indicates plastering.

Spirits.

Spirits.—Whisky, brandy, rum, gin, etc.

Whisky.—Whisky is made from malt or malt and grain, and distilled in pot-stills or patent-stills. For many years superior claims were made for the pot-still article, but these claims have been destroyed by the report of the recent Royal Commission.

In 1905 a London magisterial investigation decided that patent-still spirit alone is not whisky, and that whisky cannot be made from maize; the above report upsets this view.

The pot-still in its simplest form is a pot with a long neck over which the distilled alcohol passes when the wash or fermented mash of grain is boiled. Usually two distillations are carried out in producing Scotch whisky.

The patent-still is an arrangement of pipes and chambers through which steam is passed continuously as the wash distils. This is a cheaper process capable of a much greater output.

The Commission concluded that it would be no advantage to prohibit the use of foreign barley, and it would be too arbitrary to say that Scotch whisky should be made from malt alone, and Irish from a mixture. Maize affects the flavour, but there is no valid reason for excluding it. Patent-still whiskies are less varied than pot-still, but the same effects are produced by both kinds if taken in the same quantity and in the same strength.

Pot-still distillers admit the need of blending with patent-still whisky, unless their own spirit can be matured longer; patent-still tones down the pungent taste of the other. Cheap blends contain as little as 10 per cent. of pot-still. As whiskies used in England are usually blends, and as the patent-still is adapted for economical and larger production, and as there is no evidence that the form of still has any relation to the wholesomeness of the spirit, the Commission could not recommend that the term 'whisky' should be restricted to the pot-still variety.

Brandy is determined by the report as a potable spirit made from fermented grape-juice and from no other materials. 'British brandy' is defined as a compounded spirit prepared by a rectifier or compounder by redistilling duty-paid spirits made from grain with flavouring ingredients, or by adding flavouring materials to

such spirits; the nature of the flavouring materials is not disclosed.

True brandy is distilled wine, and was originally procured from a rich Cognac district in France. Its quality varies with the character of the grapes used, the best grapes yielding grande champagne, a genuine liqueur brandy. It is to be feared that little of the brandy sold in this country is so derived. Brandy contains, beside ethyl alcohol, volatile ethers in large amount, an important distinction from whisky. Its percentage of alcohol is about the same as that of whisky.

Alcohol.—Estimation by distillation as under Beer.

Metallic Impurities.—Pb, Cu, etc. Detection and estimation as under Water.

Fusel-Oil.—Fusel-oil is the most important impurity of spirit. It is more injurious than ordinary alcohol, and should not be permitted to exceed 0.2 per cent. (1) Shake 20 c.c. of the spirit with 2 c.c. dilute KOH. Evaporate on water-bath to 2 or 3 c.c. Cool and add 5 c.c. strong sulphuric acid. The odours of valerianic and butyric acids will be detected if fusel-oil be present. (2) Distil off four-fifths of the sample, and extract the residue with ether; allow the extract to evaporate spontaneously, and treat what is left with H_2SO_4 and sodium acetate: the odour of pear is emitted. (3) Evaporate 50 c.c. slowly over a steam-bath; carefully smell the remainder for traces of fusel-oil. (4) Decolourize a portion of the sample with animal charcoal and add a few drops each of hydrochloric acid and colourless aniline-oil. In the presence of fusel-oil a rose tint is produced in the aniline-oil.

[Tests for methylated spirit: (1) Odour; (2) a weak solution of sodium nitroprusside (1 per cent.) and ammonia, added to a mixture containing methylated spirit, give a red colour within ten or fifteen minutes.]

Estimation—Marquardt Method.—To 100 c.c. spirit add 20 c.c. $\frac{\text{N}}{2}$ NaOH, and saponify by allowing to stand overnight, or by boiling for an hour under a reflux condenser. Distil 90 c.c.; add 25 c.c. water, and distil an additional 25 c.c. Saturate the distillate with NaCl, and add saturated NaCl solution till specific gravity is 1.1. Extract the salt solution four times with CCl_4 (recently purified by boiling with sulphuric acid and potassium bichromate, and distilling)

using 40, 30, 20, and 10 c.c., respectively. Wash the CCl_4 extract three times with 50 c.c. portions of a saturated NaCl solution, and twice with the same volumes of saturated sodium sulphate solution. Now boil the tetrachloride for eight hours with 5 c.c. concentrated H_2SO_4 , 5 grammes potassium bichromate, and 45 c.c. water under a reflux condenser. Add 30 c.c. water, and distil till about 20 c.c. remain; add 80 c.c. water, and distil till 5 c.c. are left.

Neutralize the distillate to methyl orange; add phenolphthalein, and run in $\frac{N}{10}$ NaOH till neutral. One c.c. $\frac{N}{10}$ NaOH = 0.0088 gramme amyl alcohol. In the oxidation and second distillation the corks used should be covered with tinfoil.

Röse's Method.—Chloroform quickly removes fusel-oil from dilute spirit, and the presence of fusel-oil in chloroform increases the capacity of the latter for dissolving ethylic alcohol. So, therefore, if chloroform be shaken with dilute ethyl alcohol containing fusel-oil, its volume will be considerably greater than when shaken with the same volume of pure ethyl alcohol.

Dilute the spirit to be tested until its specific gravity is 0.9655 at 15°C . (30 per cent. alcohol by volume). If the sample is weaker than this it must be fortified by absolute alcohol (1 per cent. error + or - corresponds with 0.0199 per cent. by volume of fusel-oil). In the special tube place 20 c.c. chloroform, which at 15°C . reaches the lower division of the scale. Add 100 c.c. alcohol and 1 c.c. H_2SO_4 , specific gravity 1.2857. Stopper the apparatus and shake a definite number of times, say 150. Let stand for some time, and read the volume of chloroform. Submit pure alcohol of the same strength to the same process, and note the difference in volume of the chloroform. An increase 0.01 c.c. (the scale is readable to 0.01 c.c.) is equal to 0.006631 per cent. amyl alcohol.

Methyl Alcohol—*Method of Riche and Bardy.*—This method depends on the formation of methyl-anilin-violet. To 10 c.c. sample add 15 grammes iodine and 2 grammes amorphous phosphorus. Stand in iced water till action has ceased. Distil on a water-bath the methyl and ethyl iodides into 30 c.c. water. Wash with dilute NaOH to remove free iodine. Separate the heavy oily liquid which settles, and mix with 5 c.c. anilin in a flask placed in cold water. After an hour boil and add about 20 c.c. 15 per cent. soda solution. The bases rise to the top as an oily layer; float

them up with water and pipette off. Oxidize 1 c.c. of the oily liquid by heating in a glass tube at 90 °C. for eight or ten hours with 2 parts NaCl, 3 parts $\text{Cu}(\text{NO}_3)_2$, and 100 parts clean sand. Exhaust with warm alcohol, filter, and make up to 100 c.c. with alcohol. In the case of pure spirits the liquid is red, but in the presence of 1 per cent. methyl alcohol it is violet. Dilute 5 c.c. of the coloured liquid to 100 c.c. with water, and dilute 5 c.c. of this again to 400 c.c. Heat the liquid in a porcelain dish with some pure white merino wool (free from sulphur) for half an hour. If the spirits be pure the wool will remain white, but if methylated the fibre will become violet. A quantitative estimation can be made by comparing the tint with a set of standards containing known percentages of methylic alcohol.

Rum is prepared from molasses, a by-product in the manufacture of sugar, but the best varieties are obtained by fermenting the juice of the sugar-cane. One by-product—ethyl butyrate—confers upon it its characteristic flavour. Like brandy, however, much of the rum sold in this country is made from silent spirit, flavoured with characteristic by-products.

Gin is prepared by distilling and redistilling a mixture of rye and malt. In the last distillation juniper berries, salt, and hops, are added, and the product is run off into cisterns lined with white tiles, whereby colouring matters are prevented entering the spirit. The best gins are distilled in Holland; but much of the gin of commerce is concocted from silent spirit, resins, and juniper berries.

The term 'proof-spirit' is applied to a mixture of 57·06 per cent. by volume of absolute alcohol in water. It has a specific gravity of 919·8 at 15° C.

Brandy, whisky, and rum, may be 25 degrees under proof—that is, may contain 75 per cent. of the alcohol found in proof-spirit. Gin may be 35 degrees under proof—that is, may contain 65 per cent. of the alcohol found in proof-spirit.

Spirits generally contain 40 to 60 per cent. of alcohol; wines 8 to 16; beers 5 to 7.

Acidity of Spirits.—Titrate with decinormal alkali and calculate as acetic acid. One c.c. $\frac{N}{10}$ alkali = 0·006 gramme acetic acid.

Esters.—Dilute 250 c.c. of the spirit with 50 c.c. water, and distil 200 c.c. Neutralize 50 c.c. of the distillate with decinormal alkali

(phenolphthalein indicator); add $\frac{N}{10}$ alkali in considerable excess. Boil for an hour under a reflux condenser. Cool and titrate with $\frac{N}{10}$ alkali. The number of cubic centimetres $\frac{N}{10}$ alkali used in the saponification multiplied by 0.0088 = grammes esters calculated as ethyl acetate.

Furfural.—Prepare a standard furfural solution. Dissolve 1 gramme redistilled furfural in 100 c.c. 95 per cent. alcohol. Dilute 1 c.c. of this to 100 c.c. with 50 per cent. alcohol. One c.c. = 0.0001 gramme furfural. Dilute 20 c.c. of the above distillate to 50 c.c. with 50 per cent. alcohol free from furfural. Add 2 c.c. colourless anilin and 0.5 c.c. HCl, specific gravity 1.125. Make standards, from which match the tint.

Purfural is found in pot-still, but not in patent-still, spirit.

Alcohol Table.

| Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
under Proof. | Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
under Proof. |
|----------------------|------------------------------|---------------------------|----------------------|------------------------------|---------------------------|
| 1.000 | 0.00 | 100.00 | 0.973 | 23.10 | 59.52 |
| 0.999 | 0.66 | 98.84 | 0.972 | 24.08 | 57.80 |
| 0.998 | 1.34 | 97.66 | 0.971 | 25.07 | 56.06 |
| 0.997 | 2.12 | 96.29 | 0.970 | 26.04 | 54.37 |
| 0.996 | 2.86 | 95.00 | 0.969 | 26.95 | 52.77 |
| 0.995 | 3.55 | 93.78 | 0.968 | 27.86 | 51.18 |
| 0.994 | 4.27 | 92.50 | 0.967 | 28.77 | 49.60 |
| 0.993 | 5.00 | 91.23 | 0.966 | 29.67 | 48.00 |
| 0.992 | 5.78 | 89.87 | 0.965 | 30.57 | 46.44 |
| 0.991 | 6.55 | 88.50 | 0.964 | 31.40 | 44.97 |
| 0.990 | 7.32 | 87.16 | 0.963 | 32.19 | 43.60 |
| 0.989 | 8.18 | 85.65 | 0.962 | 32.98 | 42.20 |
| 0.988 | 9.04 | 84.15 | 0.961 | 33.81 | 40.74 |
| 0.987 | 9.86 | 82.70 | 0.960 | 34.54 | 39.47 |
| 0.986 | 10.73 | 81.20 | 0.959 | 35.28 | 38.18 |
| 0.985 | 11.61 | 79.65 | 0.958 | 36.04 | 36.83 |
| 0.984 | 12.49 | 78.10 | 0.957 | 36.70 | 35.68 |
| 0.983 | 13.43 | 76.46 | 0.956 | 37.34 | 34.57 |
| 0.982 | 14.37 | 74.82 | 0.955 | 38.04 | 33.32 |
| 0.981 | 15.30 | 73.18 | 0.954 | 38.75 | 32.08 |
| 0.980 | 16.24 | 71.54 | 0.953 | 39.47 | 30.84 |
| 0.979 | 17.17 | 69.90 | 0.952 | 40.14 | 29.66 |
| 0.978 | 18.25 | 68.00 | 0.951 | 40.79 | 28.52 |
| 0.977 | 19.28 | 66.20 | 0.950 | 41.32 | 27.60 |
| 0.976 | 20.24 | 64.53 | 0.949 | 41.84 | 26.67 |
| 0.975 | 21.19 | 62.87 | 0.948 | 42.40 | 25.70 |
| 0.974 | 22.18 | 61.13 | 0.947 | 42.95 | 24.74 |

Alcohol Table—continued.

| Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
under Proof. | Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
over Proof. |
|----------------------|------------------------------|---------------------------|----------------------|------------------------------|--------------------------|
| 0.946 | 43.56 | 23.66 | 0.899 | 66.25 | 16.11 |
| 0.945 | 44.18 | 22.58 | 0.898 | 66.69 | 16.88 |
| 0.944 | 44.79 | 21.50 | 0.897 | 67.11 | 17.61 |
| 0.943 | 45.41 | 20.43 | 0.896 | 67.53 | 18.34 |
| 0.942 | 46.02 | 19.36 | 0.895 | 67.93 | 19.05 |
| 0.941 | 46.59 | 18.36 | 0.894 | 68.33 | 19.74 |
| 0.940 | 47.13 | 17.40 | 0.893 | 68.72 | 20.42 |
| 0.939 | 47.67 | 16.46 | 0.892 | 69.11 | 21.11 |
| 0.938 | 48.21 | 15.50 | 0.891 | 69.50 | 21.79 |
| 0.937 | 48.75 | 14.57 | 0.890 | 69.92 | 22.53 |
| 0.936 | 49.29 | 13.63 | 0.889 | 70.35 | 23.29 |
| 0.935 | 49.81 | 12.70 | 0.888 | 70.77 | 24.02 |
| 0.934 | 50.31 | 11.82 | 0.887 | 71.17 | 24.73 |
| 0.933 | 50.82 | 10.94 | 0.886 | 71.58 | 25.44 |
| 0.932 | 51.32 | 10.05 | 0.885 | 71.98 | 26.15 |
| 0.931 | 51.82 | 9.20 | 0.884 | 72.38 | 26.85 |
| 0.930 | 52.29 | 8.36 | 0.883 | 72.77 | 27.52 |
| 0.929 | 52.77 | 7.52 | 0.882 | 73.15 | 28.19 |
| 0.928 | 52.24 | 6.70 | 0.881 | 73.54 | 28.87 |
| 0.927 | 53.72 | 5.86 | 0.880 | 73.93 | 29.57 |
| 0.926 | 54.19 | 5.03 | 0.879 | 74.33 | 30.26 |
| 0.925 | 54.66 | 4.20 | 0.878 | 74.70 | 30.92 |
| 0.924 | 55.13 | 3.38 | 0.877 | 75.08 | 31.58 |
| 0.923 | 55.60 | 2.56 | 0.876 | 75.45 | 32.23 |
| 0.922 | 56.07 | 1.74 | 0.875 | 75.83 | 32.89 |
| 0.921 | 56.54 | 0.92 | 0.874 | 76.20 | 33.54 |
| 0.920 | 56.98 | 0.14 | 0.873 | 76.57 | 34.19 |
| 0.9198 | 57.06 | Proof | 0.872 | 76.94 | 34.84 |
| | | Per Cent.
over Proof. | 0.871 | 77.29 | 34.45 |
| | | | 0.870 | 77.64 | 36.07 |
| 0.919 | 57.45 | 0.68 | 0.869 | 78.00 | 36.69 |
| 0.918 | 57.92 | 1.51 | 0.868 | 78.36 | 37.33 |
| 0.917 | 58.36 | 2.28 | 0.867 | 78.73 | 37.98 |
| 0.916 | 58.80 | 3.05 | 0.866 | 79.12 | 38.65 |
| 0.915 | 59.22 | 3.78 | 0.865 | 79.50 | 39.32 |
| 0.914 | 59.63 | 4.50 | 0.864 | 79.86 | 39.96 |
| 0.913 | 60.07 | 5.27 | 0.863 | 80.22 | 40.60 |
| 0.912 | 60.52 | 6.07 | 0.862 | 80.60 | 41.26 |
| 0.911 | 60.97 | 6.86 | 0.861 | 81.00 | 41.96 |
| 0.910 | 61.40 | 7.61 | 0.860 | 81.40 | 42.66 |
| 0.909 | 61.84 | 8.37 | 0.859 | 81.80 | 43.35 |
| 0.908 | 62.31 | 9.20 | 0.858 | 82.19 | 44.04 |
| 0.907 | 62.79 | 10.03 | 0.857 | 82.54 | 44.66 |
| 0.906 | 63.24 | 10.84 | 0.856 | 82.90 | 45.28 |
| 0.905 | 63.69 | 11.64 | 0.855 | 83.25 | 45.90 |
| 0.904 | 64.14 | 12.41 | 0.854 | 83.60 | 46.51 |
| 0.903 | 64.58 | 13.18 | 0.853 | 83.94 | 47.11 |
| 0.902 | 65.01 | 13.92 | 0.852 | 84.27 | 47.70 |
| 0.901 | 65.41 | 14.62 | 0.851 | 84.60 | 48.27 |
| 0.900 | 65.81 | 15.33 | 0.850 | 84.93 | 48.84 |

Alcohol Table—continued.

| Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
over Proof. | Sp. Gr. at
15° C. | Per Cent.
Alcohol (Vol.). | Per Cent.
over Proof. |
|----------------------|------------------------------|--------------------------|----------------------|------------------------------|--------------------------|
| 0·849 | 85·26 | 49·38 | 0·820 | 94·00 | 64·74 |
| 0·848 | 85·59 | 50·00 | 0·819 | 94·26 | 65·18 |
| 0·847 | 85·94 | 50·61 | 0·818 | 94·51 | 65·62 |
| 0·846 | 86·28 | 51·21 | 0·817 | 94·76 | 66·07 |
| 0·845 | 86·61 | 51·78 | 0·816 | 95·03 | 66·53 |
| 0·844 | 86·93 | 52·34 | 0·815 | 95·29 | 67·00 |
| 0·843 | 87·24 | 52·90 | 0·814 | 95·55 | 67·46 |
| 0·842 | 87·55 | 53·43 | 0·813 | 95·82 | 67·92 |
| 0·841 | 87·85 | 53·96 | 0·812 | 96·08 | 68·38 |
| 0·840 | 88·16 | 54·50 | 0·811 | 96·32 | 68·80 |
| 0·839 | 88·46 | 55·02 | 0·810 | 96·55 | 69·20 |
| 0·838 | 88·76 | 55·55 | 0·809 | 96·78 | 69·61 |
| 0·837 | 89·08 | 56·10 | 0·808 | 97·02 | 70·03 |
| 0·836 | 89·39 | 56·66 | 0·807 | 97·27 | 70·46 |
| 0·835 | 89·70 | 57·20 | 0·806 | 97·51 | 70·88 |
| 0·834 | 89·99 | 57·71 | 0·805 | 97·73 | 71·26 |
| 0·833 | 90·29 | 58·23 | 0·804 | 97·94 | 71·64 |
| 0·832 | 90·58 | 58·74 | 0·803 | 98·16 | 72·02 |
| 0·831 | 90·88 | 59·26 | 0·802 | 98·37 | 72·40 |
| 0·830 | 91·17 | 59·77 | 0·801 | 98·59 | 72·77 |
| 0·829 | 91·46 | 60·28 | 0·800 | 98·80 | 73·14 |
| 0·828 | 91·75 | 60·79 | 0·799 | 98·98 | 73·47 |
| 0·827 | 92·05 | 61·32 | 0·798 | 99·16 | 73·81 |
| 0·826 | 92·36 | 61·86 | 0·797 | 99·35 | 74·14 |
| 0·825 | 92·66 | 62·38 | 0·796 | 99·55 | 74·50 |
| 0·824 | 92·94 | 62·88 | 0·795 | 99·75 | 74·83 |
| 0·823 | 93·23 | 63·38 | 0·794 | 99·96 | 75·18 |
| 0·822 | 93·49 | 63·84 | 0·7938 | 100·00 | 75·25 |
| 0·821 | 93·75 | 64·30 | | | |

OTHER FOODSTUFFS.

Lime and Lemon Juices.—The Board of Trade requires that these juices shall have a specific gravity of 1·030, and shall have acidity of 6·8 per cent. citric acid. The specific gravity is determined in the usual manner; and the free acidity by titration with $\frac{N}{10}$ soda (1 c.c. = 0·007 gramme citric acid). Where sulphuric or other mineral acid is added as an adulterant, it is estimated in the usual way. These juices are generally fortified with 3 to 4 per cent. of alcohol.

Adulterants.—Tartaric acid, mineral acids, glucose, cane-sugar, invert-sugar, preservatives, coal-tar dyes. With the exception of tartaric acid, determine each of these in the usual manner.

Tartaric Acid.—Mix 20 grammes of juice with 5 grammes KCl; neutralize with KOH, and make up to 50 c.c. with water. Add 5 grammes citric acid, stir the solution, and stand overnight. Wash the precipitated acid potassium tartrate with a saturated solution of acid potassium tartrate, and afterwards two or three times with 10 per cent. KCl. Titrate hot with $\frac{N}{10}$ NaOH (1 c.c. = 0.0075 gramme tartaric acid.)

Estimation of Citric Acid—Warrington's Method.—Neutralize 15 to 20 c.c. ordinary juice, or 3 to 4 c.c. concentrated juice, with normal soda, and make up to about 50 c.c. Heat on a water-bath, and add CaCl_2 until slightly in excess of the organic acids present. Boil for half an hour, filter, and wash the precipitate with hot water. Concentrate the filtrate and washings to about 15 c.c. and add a drop of ammonia, which produces a further precipitate. Collect this on a small filter with the assistance of the previous filtrate; wash with a small quantity of hot water. Dry both precipitates; ignite at a low red heat, and titrate the ash with $\frac{N}{10}$ acid (1 c.c. = 0.007 gramme $\text{H}_3\cdot\text{Ci}\cdot\text{H}_2\text{O}$).

Vinegar.—Malt vinegar, as distinguished from wood vinegar (acetic acid and water), is made by soaking malt or malt and barley in successive quantities of hot water until the extraction is complete, fermenting the extract with yeast, and finally pumping the fermenting mass over wickerwork coated with *Mycoderma aceti* by which the alcohol is converted into acetic acid. Other bodies, such as aldehydes, acetic ether, etc., are formed at the same time.

Specific Gravity.—Determine in the usual way; specific gravity should be about 1.019.

Acetic Acid.—Dilute 10 c.c. to 50 c.c. and titrate with $\frac{N}{10}$ NaOH (phenolphthalein as indicator). (1 c.c. $\frac{N}{10}$ NaOH = 0.006 gramme acetic acid.)

Nitrogen.—Operate on 25 c.c. by the Kjeldahl process.

Phosphoric Acid.—Operate on 25 c.c. by Neumann's method.

Sulphuric Acid.—When the ash of vinegar fails to be alkaline, mineral acid has been added. Evaporate 50 c.c. of the sample to dryness with 25 c.c. $\frac{N}{10}$ NaOH, and ignite at lowest possible temperature. Add 25 c.c. $\frac{N}{10}$ HCl, heat to expel CO_2 , and filter; wash with hot water, and collect washings, and filtrate. Titrate the free acid with $\frac{N}{10}$ NaOH and phenolphthalein (1 c.c. $\frac{N}{10}$ NaOH = 0.0049 gramme H_2SO_4).

Total Solids.—Evaporate 25 c.c. to constant weight. Ignite the residue at a low temperature to obtain the ash.

A good malt vinegar contains roughly 5.5 per cent. acetic acid, 2.5 per cent extract, 0.5 per cent. ash, 0.075 per cent. P_2O_5 , 0.075 per cent. N, and has a specific gravity of 1.020.

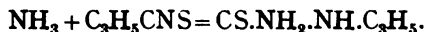
Mustard.—Mustard is derived from the seeds of the black and white mustard plants (*Brassica nigra* and *alba*). A little turmeric and coal-tar colours are usually added to the ground seeds, and sometimes foreign starches and ground chillies (small pods of Cayenne pepper). These adulterants are all harmless. Starch is readily detected by the iodine test. Turmeric becomes brownish-red under the action of ammonia. White mustard is recognised under the microscope by the hexagonal or 'infundibuliform' cells



FIG. 67.—CELLS OF CUTICLE OF MUSTARD.

of the cuticle, possessing a central ostium occupied by the so-called 'mucilage' cells.

Mustard oil is a slightly yellow refractive liquid of strong odour. It boils between 148° and 156° C., and has a specific gravity varying between 1.020 and 1.030. Colour changes to reddish-brown on exposure to light. Volatile oil of black mustard interacts with ammonia to form thiosinamine:



The oil is estimated by extracting with ether in a Soxhlet's apparatus. Good samples contain about 30 per cent. oil.

Pepper.—Black pepper is derived from the unripe berries of *Piper nigrum*, and white pepper from the ripe fruit. A transverse section of a black-pepper berry presents an external layer of cells, somewhat resembling bean starch granules, within these a layer

of elongated cells arranged transversely to the foregoing, next a reticulum containing oil globules, more internally still a layer of flask-shaped cells, and finally a central mass of angular cells containing starch.

Pepper is largely adulterated, but with substances which are harmless. These are various foreign starches, palm-nut powder, ground stones of olives, ground shells of walnuts, and occasionally chalk, clay, and brick-dust.

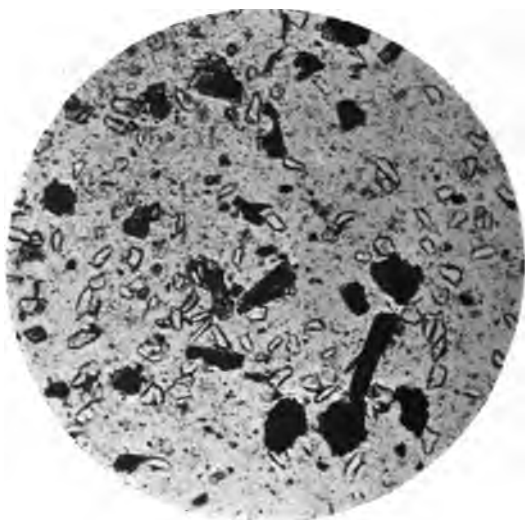
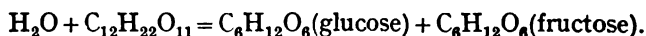


FIG. 68.—BLACK PEPPER. $\times 30$.

The ash of black pepper should not exceed 6.5 per cent., and that of white pepper should not exceed 3.5 per cent. In microscopically examining pepper, it must be remembered that, unlike mustard, pepper naturally contains starch.

Sugar.—Glucose (dextrose), or grape-sugar, and fructose (lævulose) occur in grapes and other fruits, together with sucrose (cane-sugar).

Cane-sugar probably develops first, and afterwards gives origin to the other two; this change is readily produced by hydrolysis:



Cane-sugar is widely distributed in the vegetable kingdom. It forms one of the most important foodstuffs. Little chemical energy is required to convert it into glucose (the final form of digested carbohydrates) compared with that necessary to transform starches.

Inasmuch as all carbohydrates must pass into the form of glucose before they can be utilized in the cell, and inasmuch as the greater portion of the kinetic energy of the body is liberated in the combustion of glucose, especial interest centres round this body.

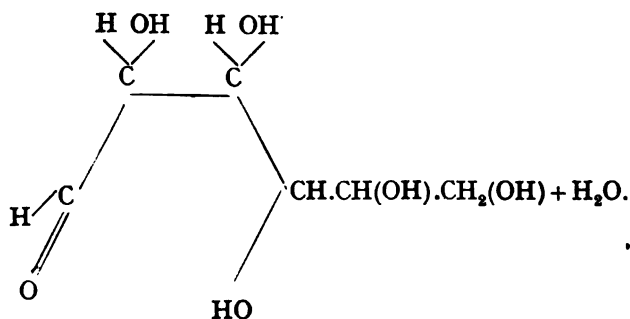
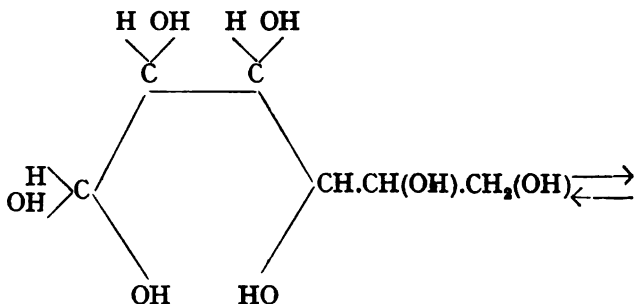
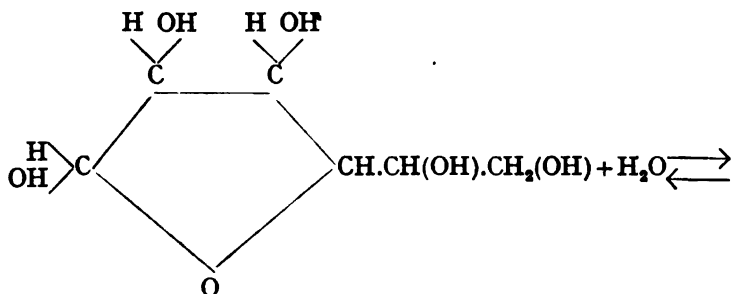
In the alimentary canal the higher carbohydrates are transformed into the simpler by hydrolysing enzymes. The simpler carbohydrates are all represented empirically by the formula CH_2O . The simplest is CH_2O , formaldehyde, probably first produced in the plant from CO_2 and H_2O by the influence of sunlight in the presence of chlorophyll. Glucose has been synthesized from formaldehyde.

Glucose is readily prepared by hydrolysing lactose, maltose, starch, and cellulose. When treated with HI it loses all its oxygen, and is converted into $\text{C}_6\text{H}_{13}\text{I}$, a derivative of hexane, $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3$. Five of its six atoms of O occur in the alcoholic (OH) form and one in the aldehydic. Owing to its stability it is assumed that each of the hydroxyls is linked to a different carbon atom; its constitutional formula may be written $\text{CH}_2\cdot\text{OH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CHO}$. Since glucose is much less active than its hydroxy-aldehydic character indicates, and by reason of its general reactions, it has been assumed (and the assumption has met with the highest support) that four of its six carbon atoms and one oxygen atom are arranged in the form of a pentagonal ring, and that when by hydrolysis the ring is broken, it passes into the aldehydic form, as shown on p. 272.

The four carbon atoms in the ring and also that immediately contiguous to the ring on the right are asymmetric, so that the ring form may be written in either of two ways, constituting an α - and a β -glucose. This conception of two stereoisomeric forms of glucose explains better than any other the gradual change (sometimes increase, but generally decrease) in optical rotation which occurs in the freshly dissolved substance until a constant value is obtained. Glucose, like the other aldoses and ketoses, is a reducing agent—*i.e.*, it greedily absorbs oxygen from alkaline solutions of metallic oxides. Alkaline copper solution on warming becomes

red cuprous oxide, and ammoniacal silver solutions form a metallic mirror.

The reaction of glucose and other sugars to excess of phenylhydrazine in acid solution enabled Fischer to demonstrate the



chemistry of the carbohydrates. If glucose be heated with excess of phenylhydrazine and acetic acid, the insoluble osazone separates after some time.

Glucose, mannose, and fructose give the same phenylosazone. Glucose is transformed into the hexahydric alcohol sorbitol by reduction with sodium amalgam; in like manner mannose is converted into mannitol, and galactose into dulcitol. Glucose is oxidized to gluconic acid by bromine; the aldehyde group becomes carboxyl.

Oxidation by nitric acid transforms glucose into bibasic saccharic acid.

Glucose is rapidly oxidized in the animal body under normal conditions to CO_2 and H_2O ; but when combined with such bodies as chloral and camphor, the aldehyde end of the glucose molecule escapes, and oxidation takes place at the other extremity, producing glucuronic acid, which is excreted in the urine.

The power of removing toxic substances from circulation in combination with glucose appears to be common to both the animal and vegetable kingdoms. The salts of glucuronic acid in animals are analogous to the glucosides in vegetables.

Disaccharides consist of two six-carbon atom groups joined by an oxygen atom, and are consequently analogous to the simple glucosides. On hydrolysis they split into their constituent hexoses, which may be either aldoses or ketoses. One hexose reduces cupric salts, forms an osazone, and displays mutarotation like glucose; the other fails in all these respects. Maltose and lactose belong to the first class.

Rubner's isodynamic law—viz., 'in dietaries, fats and carbohydrates are mutually replaceable in definite proportions, the sole limitation being that imposed by the digestive organs'—is, in the light of recent work, only partially true. This method of assessing the value of a dietary on its caloric value, whilst of admitted use, is misleading. It takes no account of the chemical form of foodstuffs. It is now certain that in man at least there must be a constant supply of carbohydrate circulating in the body fluids. Even in advanced starvation the glucose content of the blood varies but little from that of the normal. If fat be largely substituted for carbohydrate, the output of N rises, and this rise of N is due to the demand of the organism for sugar which is extracted from amino-acids—in a word, undue katabolism takes place in the most important tissue constituents. This protein breakdown cannot be

inhibited by the administration of fat. Again, of the two stereoisomeric forms of glucose, one is preferentially metabolized by the animal organism. It may be fairly stated that of isomeric synthetic foodstuffs that form alone is assimilated and oxidized that occurs in nature. A limit is thus set to the synthesis of foods.

Manufacture of Cane-Sugar.—The juice of the cane is extracted by the rollers of the crushing-mills, and is freed from proteins, acids, etc., by 'defecation'—coagulation of albumins, etc., and neutralization with milk of lime. When the impurities are removed as a scum, the juice is subjected to evaporation and crystallization. The raw sugar is thus separated from the mother-liquor, or molasses.

But sugar is prepared by digesting sliced beets with warm water, and then clarifying as above.

Sugar is refined by clarifying it with various reagents—lime, clay, acid phosphate of calcium, blood, etc. The syrup from which the purified sugar is crystallized is sold as 'golden syrup.'

Sugar is met with in all conditions of purity:

Raw sugars (brown sugar, etc.) contain from 0.5 to 5 per cent. of moisture; refined sugars below 0.5 per cent.

The ash consists of lime, oxides of K and Na, alumina, silica, and runs from 0.05 to 2 per cent. Sometimes brown sugars contain sand.

Aniline dyes are employed to colour sugars. Such samples will turn pink on addition of HCl and a little heat.

The natural colour of sugar is not extracted with alcohol; if the dyed sample be extracted with this reagent in the absolute form, and a little wool previously mordanted with aluminium acetate placed in the solution, the wool will be coloured yellow. Further, on examination of the crystals with a microscope, the dye will be found unequally distributed.

Beet-sugar is bleached with SO_2 , or bone black, and afterwards dyed with ultramarine.

Cane-sugar (sucrose) is dextrarotatory, but invert-sugar (the product of hydrolysis) is lævorotatory (fructose is more lævorotatory than dextrose is dextrorotatory).

Maltose is produced by the action of diastase on starch. It crystallizes in small needles, is dextrorotatory, and displays mutarotation. It reduces Fehling's solution, forms a phenylosazone,

and reacts in other ways like glucose. When hydrolysed by acids, it forms two molecules of glucose; maltose is fermented only by maltase. The enzymes diastase, invertase, lactase, and emulsin fail to affect it. It has been therefore considered as a glucose- α -glucoside, since α -glucosides only are hydrolysed by matase.

The sugars are estimated by Fehling's solution, or the Pavy modification, or by the polarimeter. Each polarimeter works on its own 'normal weight of sugar'—the amount of sucrose dissolved in 100 c.c. of water, which produces a deviation of 100° on the sugar scale, or 66.5 angular degrees. The Laurent instrument takes 16.19 grammes, the Soleil and Schmidt instruments 26.05 grammes.

Fehling's solution may be used volumetrically, as already described, or gravimetrically, in which case to the boiling Fehling is added a measured quantity of sugar solution insufficient to reduce all the copper; the Cu_2O precipitate is washed, dried, and weighed, or reduced to metallic copper and weighed.

The amount of cane-sugar in a sample may be determined by transforming it to grape-sugar, and estimating the amount of the latter by Fehling's method. The inversion is performed by heating a quantity of the sugar with about a tenth of its bulk of strong hydrochloric acid for ten to fifteen minutes on a water-bath. The inverted fluid before titration is neutralized with sodium carbonate.

The other items of the analysis are carried out by the usual methods.

Tea.—Tea consists of the dried leaves of several varieties of *Camella thea*. The leaves are prepared for microscopical examination by soaking in water until they assume their original shape, when they are carefully dried between layers of blotting-paper and mounted on large microscopic slides in Farrant's solution. The leaf is elliptical, and possesses an emarginate apex. The ribs form a looped network, arranged symmetrically on either side of the mid-rib, and approaching, but not quite reaching, the edge of the leaf, thereby leaving a clear marginal space. The margin is serrated from a point near to the apex to another point some little distance from its attachment to the stalk; the point of each serration is surmounted by a small spine.



When a leaf is immersed in a warm 20 per cent. solution of NaOH, mounted on a slide, and the cover-slip pressed down, long



FIG. 69.—CUTICLE OF TEA-LEAF. $\times 200$.



FIG. 70.—IDIOLASTS IN SECTION OF
TEA-LEAF. $\times 160$.

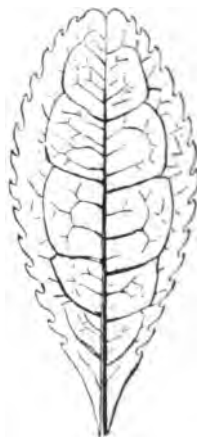


FIG. 71.—TEA-LEAF.

tenacious, branched cells, termed idioblasts, are to be seen. These cells do not occur in any other leaves likely to be mistaken for tea.

Black and green teas differ only in their mode of preparation.

Composition of an Average Sample of Black Tea :

| | | | | | | |
|--------------------------------|----|----|----|----|----|------|
| Water | .. | .. | .. | .. | .. | 8.2 |
| Thein | .. | .. | .. | .. | .. | 3.2 |
| Tannic acid | .. | .. | .. | .. | .. | 16.4 |
| Pectin, cellulose, chlorophyll | .. | .. | .. | .. | .. | 40.6 |
| Proteins | .. | .. | .. | .. | .. | 18.0 |
| Alcoholic extract | .. | .. | .. | .. | .. | 7.3 |
| Ash | .. | .. | .. | .. | .. | 6.3 |

Of these constituents, the most important are the alkaloid thein and tannic acid, for these, with 0.5 per cent. of volatile oil, produce the characteristic effects of tea.

Indian and Ceylon teas are richer in all three constituents (thein, tannin, volatile oil) than China teas; and green tea is richer in tannic acid than black; but the amount of thein is about the same in both.

If tea be infused for five minutes in the usual manner, about one-fourth of the weight of the leaf goes into solution. The thein is so soluble that it passes into solution almost immediately, but the tannic acid requires some time to dissolve. There is less tannic acid after three minutes' solution than after five, and less after five than after ten; after a longer interval there is not very much change, as practically all the soluble materials have been extracted in ten minutes; therefore the less tannic acid desired, the shorter should be the time of infusion. The method of infusion is, from a health point of view, more important than the character of leaf used. First, the water should be of medium hardness, well aerated, and just brought to the boiling-point, when tea is infused. If the water be too hard, the lime and other salts present interfere with the extraction of some of the constituents of the leaf; if, on the other hand, it be too soft, an unpleasant, bitter material is extracted. Infusion should last for about three minutes, as not only does prolonged infusion extract too much tannic acid, but it also dissipates the volatile oil to which the fragrance of tea is largely due. A further point of import is that too much leaf should not be infused; considerably less than the proverbial teaspoonful per head, when properly infused, is sufficient to produce the most fragrant and pleasant beverage. The addition of milk to tea, through the proteins that it contains, tends to precipitate some of the tannic acid. Sugar adds considerably to its nutritive value.

The average proportions of the three active ingredients in ordinary teas in use at the present day are roughly as follows:

| | | | | | |
|--------------|----|----|----|----|------------------|
| Thein | .. | .. | .. | .. | 2 to 4 per cent. |
| Tannin | .. | .. | .. | .. | 10 to 12 „ |
| Volatile oil | .. | .. | .. | .. | 0.5 „ |

Adulteration.—Admixture with foreign leaves, such as elder-leaf, sloe-leaf, and the leaf of the willow, has been effected. A low-power microscope readily detects any of these (the leaves most commonly employed) from the tea-leaf, as none of them possess an

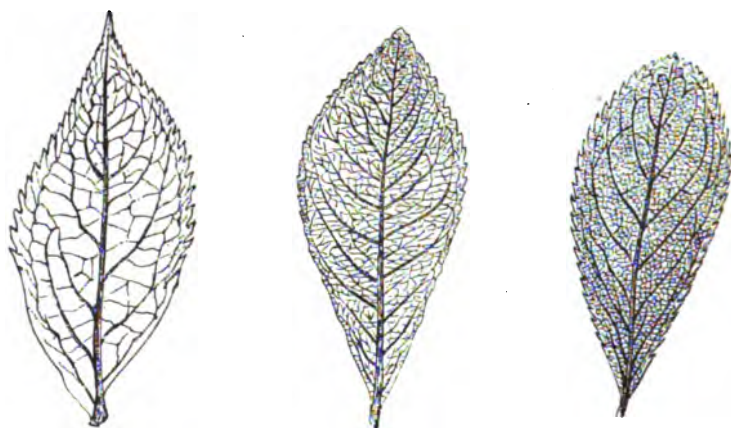


FIG. 72.—ELDER-LEAF. FIG. 73.—WILLOW-LEAF. FIG. 74.—SLOE-LEAF.

emarginate apex, nor do their systems of venation leave a clear space within the margin. The employment of infused leaves has been practised, and it may be sometimes difficult to distinguish certain prepared leaves from the genuine leaf. Various chemicals have been used to colour and 'face' previously infused leaves, such as turmeric, sulphate of lime, Prussian blue, and black-lead. Old leaves have been worked up with sand and gum, and re-rolled. It may be quite impossible to detect small quantities of such leaves in adulterated samples, since genuine teas vary much in the relative amounts of their constituents. The ash of tea should fall between 4.7 and 6.2 per cent., and the ash soluble in water should not fall below 30 per cent. of the total ash. Reference to these

figures will often assist in arriving at a conclusion as to whether or not used leaves have been added. In faced teas the ash is sometimes 10 per cent., whilst in exhausted teas it is rarely more than 0.8 per cent. The weight of the ash is determined by carefully incinerating a convenient quantity of tea—say 5 grammes—in a platinum dish, and weighing the greenish-grey mineral residue. This is the total ash. The contents of the dish are next treated with boiling water, and thrown on a Swedish filter-paper. The insoluble portion is thoroughly washed on the filter with water,

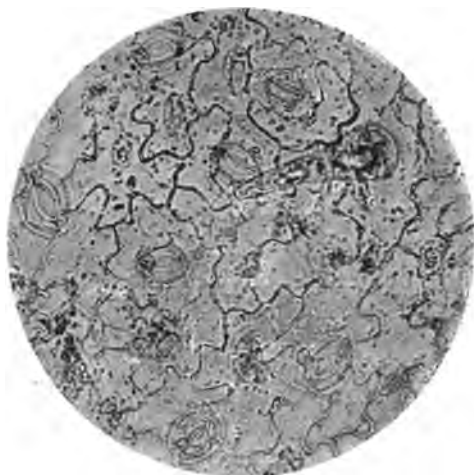


FIG. 75.—CUTICLE OF TOBACCO-LEAF. $\times 200$.

reignited, and weighed. The difference between this and the previous weight represents the soluble ash.

Estimation of Thein.—This estimation is also of value where the presence of exhausted leaves is suspected. Extract 5 grammes of finely powdered tea with 300 c.c. of boiling water, in a flask fitted to a reflex condenser, for two hours. Repeat the extraction a second and third time, allowing two hours in each case. Collect the three extracts in a beaker; add neutral lead acetate, and boil for ten minutes. Filter. Free the filtrate from lead by passing through it H_2S . Evaporate to dryness on a water-bath with some freshly ignited magnesia and clean sand, and when thoroughly

dry, powder, and carefully extract with chloroform in a Soxhlet apparatus. Evaporate the chloroform extract, and boil the residue with water. Filter. Evaporate the filtrate to dryness; continue the drying at a temperature under 100° C. Weigh, to obtain the amount of thein in 5 grammes of tea. Thein under the microscope appears as long, white, silky needles. If the preliminary extractions are not thoroughly performed, some of the thein will remain in the tissues of the leaf.

A short and rapid method of detecting thein in tea-leaves is the following: Take two watch-glasses of the same size, and place in one a small quantity of tea, and cover with the other. Mount the pair on a wire gauze over a small Bunsen flame. In five minutes the upper glass will exhibit numerous drops of moisture; in ten minutes some fine needles of thein will be seen; and in fifteen minutes a thick crop of fully formed needles will have condensed on the watch-glass. Exhausted leaves produce no such crystals. If the watch-glass be floated on cold water, crystallization is hastened.

Catechu is added to tea to produce a semblance of richness to the infusion. When present in quantity, it may be detected by precipitating an infusion of tea with neutral lead acetate and filtering. Five c.c. of the filtrate, when mixed with 2 drops of dilute ferric chloride solution, assume a green colour, which ultimately settles as a darker precipitate.

Estimation of Tannin: Proctor's Modification of Lowenthal's Method.—Ascertain how much permanganate of potassium is reduced by tannic acid, and other readily oxidizable substances in the infusion. Precipitate the tannin by gelatin, and once more determine the amount of permanganate reduced. The difference represents the quantity of permanganate decomposed by tannin.

Boil 5 grammes powdered tea in 400 c.c. water; cool, and make up to 500 c.c. To 10 c.c. filtered, if necessary, add 25 c.c. indigo carmine solution (6 grammes indigo and 50 c.c. concentrated H_2SO_4 per litre), and about 750 c.c. water. Run in from a burette potassium permanganate solution (about 1.33 grammes per litre) a little at a time, stirring the while till the colour becomes light green, then drop by drop till the colour changes to bright yellow or faint pink at the rim. Let the number of c.c. permanganate used = a .

Mix 100 c.c. of the clear infusion with 50 c.c. gelatin solution (25 grammes gelatin soaked for an hour in saturated NaCl solution, heated till dissolved, cooled, and made up to a litre), and 100 c.c. of a solution consisting of 975 c.c. saturated NaCl, and 25 c.c. concentrated H_2SO_4 ; add 10 grammes powdered kaolin, and shake well in a stoppered flask. When settled, decant the clear fluid on a filter, and afterwards bring the precipitate on the filter. To 25 c.c. of the filtrate, corresponding to 10 c.c. of the original infusion, add 25 c.c. indigo carmine solution (6 grammes indigo carmine and 50 c.c. concentrated H_2SO_4 per litre) and 750 c.c. water, and titrate with permanganate as above.

Let the number of c.c. permanganate used = b .

Now a = permanganate required to oxidize all oxidizable substances present, and b = quantity of permanganate required to oxidize substances other than tannin. Therefore the difference $a - b$ = permanganate required to oxidize the tannin. Titrate the number of c.c. permanganate represented by $a - b$ against $\frac{N}{10}$ oxalic acid. Assuming that 0.063 gramme oxalic acid = 0.04157 gramme tannin (gallotannic acid), the amount of tannin is readily calculated.

Coffee.—Coffee is derived from *Coffea arabica*. The bean is enclosed in an outer layer of fruit like the stone in a cherry, and consists of two symmetrical halves faced together, and covered by a husk. The external pulp is removed by fermentation, and the beans are dried in the air; later, the husk is separated by rolling. Many varieties of bean are to be found, the finest of which is Mocha. The beans must be roasted in order to prepare the beverage. The composition of raw and roasted Mocha coffee-beans is as follows:

| | Raw. | Roasted. |
|--------------------------------|----------|----------|
| Caffein | 1.08 .. | 0.82 |
| Caffeic acids | 8.46 .. | 4.74 |
| Sugar | 9.55 .. | 0.43 |
| Alcoholic extract | 6.90 .. | 14.14 |
| Fats.. .. . | 12.60 .. | 13.59 |
| Legumin, dextrin, cellulose .. | 48.69 .. | 60.09 |
| Moisture | 8.98 .. | 0.63 |
| Ash | 3.74 .. | 4.56 |

The roasting of coffee dissipates a small quantity of caffein and about 10 per cent. of fat, and produces an oil—caffeol—to which

the aroma of roasted coffee is due. It precipitates the albumins and separates some carbon.

Caffein is almost identical chemically with thein, but whilst thein is combined with tannin in the form of a tannate, caffein is combined with an acid allied to tannin (caffetan acid), which is not particularly astringent, does not coagulate gelatin, does not precipitate alkaloids (quinine, etc.), and gives a light-green coloration

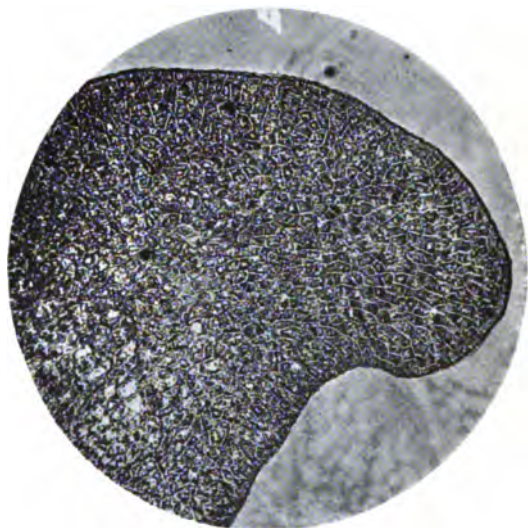


FIG. 76.—COFFEE BERRY. $\times 30$.

with Fe_2Cl_6 instead of the thick black liquid produced by thein and tannin.

Thein tannate is not very soluble in cold water, but easily soluble in hot. The caffein compound is readily soluble in cold water. When coffee infusion is saturated with $(\text{NH}_4)_2\text{SO}_4$ a precipitate is obtained which contains a small proportion of the total caffein in the free state; in tea infusion similarly treated nearly all the thein is precipitated.

The tea compound is precipitated with weak acids, and presumably by the acid of the gastric juice, and is accordingly not absorbed till it reaches the alkaline small intestine. The coffee compound is

soluble in both acids and alkalies, and is absorbed from both stomach and small intestine.

The food value of coffee is very small. It diminishes nervous fatigue somewhat, and thus assists muscular contraction. It is in some degree an antidote to alcohol.

Adulteration of Coffee.—The principal foreign substance found in coffee is chicory, a preparation of the root of the wild endive. Although the sale of chicory is allowed, and its admixture with coffee is very general, it is fraudulent to add it to samples sold as

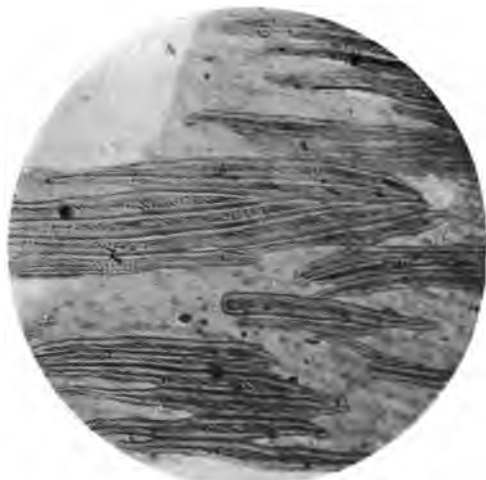


FIG. 77.—GROUND COFFEE, SHOWING CELLS OF TESTA. $\times 100$.

pure coffee. The quantity of chicory added to coffee varies very much, reaching sometimes 90 per cent. of the whole. It imparts a slightly bitter flavour to coffee, but is used principally to blacken and thicken it. Chicory is easily distinguished by the following characters: (1) Its odour is very different to that of coffee. (2) Roasted chicory sinks in water rapidly, whilst roasted coffee floats for some time, and sinks slowly, owing to the oil in the coffee preventing the particles being readily moistened. Moreover, the sediment of the coffee remains hard, whilst that of chicory is soft. (3) The specific gravity of a 10 per cent. infusion of dried chicory in water, raised to the boiling-point, maintained thereat for thirty

seconds and filtered, is rarely below 1,018, and averages about 1,022. The specific gravity of a coffee infusion prepared in the same manner is never higher than 1,010, and averages 1,008. Other and rarer adulterants, such as ground carrots, turnips, etc., will give infusions of specific gravities of 1,015 and over. (4) Microscopic examination of the powder will demonstrate the characteristic dotted and lacteal ducts of chicory, whereas in coffee portions of the membrane or testa lining the berry, and containing the characteristic spindle cells, will appear, as also endosperm cells. (5) All foreign bodies added to coffee are devoid of caffeine. (6) If to a 5 per cent. infusion of pure coffee is added a slight excess of basic lead acetate, a precipitate falls, leaving a colourless supernatant fluid: the corresponding supernatant fluid in chicory is coloured.



FIG. 78.—LACTEAL VESSELS
OF CHICORY. $\times 100$.

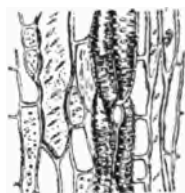


FIG. 79.—DOTTED VESSELS
OF CHICORY. $\times 100$.

When acorns, potatoes, sago, etc., are mixed with coffee, microscopic examination will detect their starch granules. Infusions of coffee and pure chicory are not blued by iodine. Caramel may be detected by its shining particles when viewed with a hand lens, as they stand out in contrast with the dull particles of coffee; also by its ready solubility in water.

Various artificial coffee-beans containing little or no real coffee have been found at times upon the market. Coffee extracts are deficient in caffeine.

Estimation of Caffein.—The method described for the estimation of them may be used. An alternative method is the following: Moisten 10 grammes finely powdered coffee with 2.5 to 3 c.c. water; stand for half an hour. Extract with CHCl_3 for three hours in a Soxhlet. Evaporate the extract. Treat the residue of fat and

cafein with hot water; filter through a cotton plug, and wash with hot water. Make up the filtrate and washings to 50 c.c. Pipette off 40 c.c., and extract four times in a separator funnel with CHCl_3 . Evaporate; dry the caffein at 100° , and weigh. Calculate the percentage.

Cocoa.—Cocoa is prepared from the seeds of a cucumber-like fruit—*Theobroma cacao*. The seeds are separated from the fruit, heaped together for some days, and allowed to ferment, which modifies their bitterness and darkens their colour. They are next roasted, when the symmetrical halves of the seed separate as cocoa-nibs on being submitted to pressure in a machine. The nibs may be sold in this form, or they may be ground between hot rollers. In the latter case the fat is melted, and the products of grinding are consequently reduced to a fluid. A considerable portion of the fat is removed by pressure, and the remainder, having been run into moulds, and thus converted into solid slabs, is once more ground and sold as a powder. Strictly speaking, cocoa is not soluble in water; the Dutch manufacturers add alkalies to it, which saponify the fat and somewhat soften the fibres of the cocoa.

Composition of Cocoa as Raw Nibs:

| | | | | | | |
|------------------------------|----|----|----|----|----|-------|
| Fat | .. | .. | .. | .. | .. | 50.44 |
| Starch | .. | .. | .. | .. | .. | 4.26 |
| Proteins | .. | .. | .. | .. | .. | 13.20 |
| Various astringents | .. | .. | .. | .. | .. | 6.71 |
| Gum and cellulose | .. | .. | .. | .. | .. | 8.57 |
| Other non-nitrogenous bodies | .. | .. | .. | .. | .. | 5.80 |
| Colouring matter | .. | .. | .. | .. | .. | 2.20 |
| Alkaloid | .. | .. | .. | .. | .. | 0.84 |
| Water | .. | .. | .. | .. | .. | 5.23 |
| Ash | .. | .. | .. | .. | .. | 2.75 |

The chief alkaloid of cocoa is theobromine (dimethyl-xanthin), a body closely related to caffein. In the commercial powder the 50 per cent. of fat is reduced to 30, or under.

Adulterants.—Foreign starches, which can be more or less easily detected by the microscope. Alkalies are frequently added in considerable quantities for the purpose of increasing the solubility of the cocoa. A determination of the ash, which in unadulterated varieties rarely exceeds 4 per cent., will assist in detecting such additions.

Chocolate is ground cocoa from which the fat has, or has not, been removed. Sugar, starch, and various flavouring materials are added, and the whole melted and thrown into moulds, or prepared for distribution in other ways.

Theobromine may be estimated thus: Remove the fat and caffen by petroleum spirit, and dry the extract on the water-bath. Boil this extract in water for a considerable time. Next extract the residue not affected by petroleum with chloroform for several hours in a Soxhlet apparatus. Drive off the chloroform on a water-bath, and boil the extract several times with water. Mix the two extracts in water, and evaporate to dryness in a platinum dish. Weigh the residue as theobromine.

CHAPTER XII

DISINFECTANTS

UNTIL comparatively recent years no very marked distinction was made between disinfectants, antiseptics, and deodorants; and this statement applies not only to the lay public, but also to sanitarians. The explanation is that, through lack of definite and exact experimental knowledge concerning the physical and chemical nature of the work to be done in disinfection, and of the agents employed therein, quite erroneous views were held upon both. Prior to the days of origin of bacteriology, a disinfectant included, besides certain physical conditions, any body capable of destroying infective or putrefactive matter, especially the noxious odours connected with putrefaction. The disposal of dead and putrefying animal and vegetable substances, more especially the bodies of dead animals and the human subject, has been a matter of special interest to man from his earliest days, as witnessed by the manner in which it is interwoven with the most sacred religious rites of the oldest nations. That important evils in the form of disease arose from lack of proper attention to such disposal was understood, but of the mode of production of such conditions nothing was truly known until bacteriology demonstrated the existence of putrefactive and pathogenic micro-organisms.

A disinfectant is a germicide or destroyer of germs. An antiseptic is a body that exerts an antagonistic or inhibitory influence on germs, and is not necessarily a disinfectant. A deodorant may possess neither antiseptic nor disinfectant properties.

Koch showed in 1881 how bacteria and their spores can be employed in the scientific study of disinfection. Later Krönig and Paul pointed out that the power of a disinfectant solution depends on certain properties inherent in the salts in solution, and the nature

of the solvent employed. Still later Bechold and Ehrlich demonstrated certain relations which exist between chemical constitution and disinfectant action, and Bechold published his views on the relations which exist between disinfection and the chemistry of the colloids.

We have known for some years that certain relations exist between the constitution of chemical substances and their physiological action. Antipyrin, for example, owes its analgesic properties to the presence of the organic radical methyl (CH_3). The introduction of a second methyl group forms a new body possessing the same pain-allaying properties in a greatly increased degree.

Desgrez pointed out in 1911 that non-saturation of the molecule increases the toxicity of the nitriles, and in a proportion greater as the saturation is less. The corresponding amides are subject to the same law. The germicidal power of an organic compound is directly proportional to the number and kind of certain radicals (phenyl, methyl, naphthyl), or, under certain conditions, of halogens (Cl, Br, I), found in the body. The germicidal activities of such radicals differ widely amongst themselves—*e.g.*, the group phenyl (C_6H_5) is about five times more energetic against certain bacteria than methyl (CH_3). Again, oxygen combined with carbon and hydrogen, and even with nitrogen, increases the bactericidal power of the compound. Nitrogen combined with one or two atoms of hydrogen always lowers antiseptic power. The substitution in an amide group of an antiseptic group (phenyl, naphthyl, etc.) immediately raises the bactericidal powers of the compound. By the accumulation of phenyl groups large increase in germicidal powers has been conferred on several compounds. Bechold and Ehrlich found that the introduction of sulphonic groups, on the other hand, lowers germicidal power. Schöller and Schrauth have shown that the introduction of halogens (Cl and I) in the benzene nucleus of oxymercuribenzoate of soda notably augments the disinfectant power of this body. But after a certain amount of halogen has been incorporated, further additions fail to raise it. Bechold and Ehrlich, working with a phenyl group, introduced successively one to five atoms of bromine. The disinfectant powers of these compounds for staphylococci and streptococci increased until three

atoms were reached, remained constant for the fourth, and diminished with the addition of the fifth. For *B. coli* they found that the maximum efficiency was reached with the second bromine atom.

In 1910, working with a phenyl group, the author was able to raise the germicidal efficiency for *B. typhosus* 20 per cent. by the incorporation of a small amount of chlorine, and 60 per cent. by saturation.

The action of germicidal agents increases with duration of contact, and also with increase of concentration. Working with anthrax spores and carbolic acid, Koch showed that in order to produce sterility, it was necessary to employ a 1 per cent. solution of the disinfectant for seven days, a 4 per cent. solution for three days, and a 5 per cent. solution for two days.

In 1889 Fraenkel and Henle drew attention to the fact that the higher homologues of phenol contained in the creolins of those days are more powerful germicides, and, being much more insoluble, are less toxic than phenol. Fraenkel and Behring at this time tested various disinfectants, using Koch's silk threads impregnated with anthrax spores; but after removing the threads from the disinfectant fluids, they transferred them to peptone bouillon, instead of solid media, as Koch had done.

In 1897 Krönig and Paul used, instead of threads, small sterile Bohemian garnets of uniform size, which they shook in the emulsion of anthrax bacilli or spores, staphylococci, etc. From time to time a definite number were taken out, and after the disinfectant had been removed by washing, these were well shaken in a measured quantity of water to remove the spores; a fractional amount of the washings was plated, and the number of germinating spores counted.

In 1907 Madsen and Nyman confirmed Krönig and Paul's work, and also the conclusion that had already been drawn from their figures by Ikeda—viz., that the disinfection of anthrax spores proceeded after the manner of a unimolecular chemical reaction, in which the velocity of chemical change at any instant is proportional to the active mass of reacting substance present at that instant. If for *concentration* (mass of reacting substance) there be

substituted *number of surviving spores*, the unimolecular reaction equation—

$$-\frac{dC}{dt} = KC$$

becomes—

$$-\frac{dN}{dt} = KN.$$

They showed that the disinfection of anthrax spores by heat conformed to the same equation.

Reichert in 1909 showed that heat-coagulated serum absorbed phenol from aqueous solution in amount directly proportional to concentration. He demonstrated further that the addition of a neutral salt like sodium chloride increased both the quantity of phenol absorbed and its germicidal power.

Cooper, in 1912, proved that egg albumin and gelatin absorb phenol and metacresol according to the partition law; and that when a certain phenol concentration is reached, the proteins are precipitated, whereby they take on a greatly increased capacity for absorbing phenol. The precipitation of gelatin by phenol is reversible, and that of egg albumin irreversible. Certain polypeptides are not precipitated by strong solutions of phenol. Cresols precipitate proteins in lower concentrations than phenol. The absorption of cresols and phenol by proteins is about the same. It appears that the inclusion in the benzene ring of the radical methyl (CH_3) increases both protein-precipitating and germicidal powers, but produces no change in the initial absorption of phenol by protein; hence it is argued that selective germicidal action is determined by the phenol-concentration at which particular proteins are precipitated, and that the disinfectant action of phenol is a mechanism similar to that of heat.

Watery solutions of antiseptics and disinfectants are more powerful than alcoholic, ethereal, and other solutions in which electrical dissociation is feeble. Koch showed that anthrax spores are not destroyed by 5 per cent. phenol in oil in 100 days, nor by the same percentage in alcohol in 70 days, whilst 5 per cent. concentration in water kills in 48 hours. These remarks apply to iodine, thymol, salicylic acid, and other bodies.

Gaseous disinfectants, such as chlorine, formaldehyde, etc.,

require a certain amount of water, or humidity of atmosphere, for the development of their activities. This is in accordance with the fundamental principle that chemical reactions take place in solution. Antiseptics dissolved in anhydrous alcohol have as a rule no more disinfectant action on bacteria than that due to the alcohol dissolving them, which, owing to its dehydrating properties, possesses a germicidal action analogous to that of desiccation by air. It has been shown that in a mixture of water and alcohol osmotic currents are established between the bacterial cells and the menstruum. Thus, whilst in pure alcohol bacteria contract, owing to dehydration, in a mixture of alcohol and water they swell, thereby offering an entrance to disinfectants.

In the case of germicides insoluble or but slightly soluble in water, as salicylic acid, thymol, etc., activity may be secured by associating them with bodies which dissolve them, or by incorporating them in certain chemical groups, such as sulpho groups.

The metallic ion appears to be the true agent of disinfection in metallic salts. These are found in liquids other than water (alcohol, ether, etc.) in a state of feeble electric dissociation; whilst in water free ionization takes place. Hence the superiority of watery preparations of salts as disinfectants.

Germicidal power is accordingly proportional to the intensity of ionization. Such an assumption permits us to explain a number of experimental facts, but not all. It enables us to conceive how the action of a disinfectant solution is inhibited by the addition of one or more extraneous substances. The decrease in activity of a solution of perchloride of mercury by the addition of sodium chloride is evidently due to a modification of dissociation. A quantity of free ions is employed for the dissociation of the sodium chloride, and consequently a less number of mercury ions remains for disinfection. The addition of acids to solutions of perchloride of mercury acts in the same manner.

Again, the germicidal powers of acids do not depend on their chemical energy, but on the degree of ionization to which each can be individually subjected. Sulphuric acid can displace nitric and hydrochloric acids by reason of its greater chemical energy; but its germicidal activity is less than that of either by reason of its

smaller electro-chemical dissociation, wherein fewer ions are liberated.

Soda, potash, and ammonia are germicidal in proportion to the concentration of the OH ions.

Whilst the addition of certain substances hinders the action of certain disinfectants by modification of dissociation, in other cases disinfectant action is assisted by such additions.

Phenol appears to act in disinfection as a molecule and not as an ion; phenylate of sodium, which is readily dissociated, has a much less germicidal value than phenol.

When a reaction takes place in a heterogeneous system, certain changes other than purely chemical occur. Since the reacting bodies are not uniformly distributed, one is compelled to travel a certain distance to come into contact with the other; diffusion is therefore a preliminary stage of the reaction. At the interfaces where the phases are in contact, there is an accumulation of surface energy. It is known that chemical and other forms of change will take the form of increase of concentration at a surface when the potential of any form of energy at that surface can be diminished by the change. This concentration of bodies on the surfaces of contact between the phases of heterogeneous systems where such potential is diminished is known as 'adsorption.'

If at this stage no purely chemical reaction occurs, the process stops; but if chemical reaction takes place, its velocity in consonance with the law of mass action is a function of the amount adsorbed, and is much greater than if no surface condensation had taken place. Adsorption undoubtedly plays a large part in many forms of disinfection, and confers upon emulsions as contrasted with solutions considerable advantages.

Bacteria present an enormous surface development. If, then, we place in contact an emulsion of bacteria and a solution of an antiseptic, the dissolved substance will tend to concentrate on the surface of the bacteria more or less strongly according to their individual nature.

We know that the same substance is a better germicide in aqueous solution than in alcoholic; we also know that adsorption phenomena are much more intense in aqueous than in alcoholic solution.

Those organic radicals which possess large germicidal powers,

such as phenyl, naphthyl, etc., influence adsorption largely; whilst other radicals which are destitute of germicidal action, such as certain sulpho-compounds, are but little adsorbed.

But ionization and adsorption do not represent the whole of the phenomena of disinfection. True chemical action must supplement these preliminary stages. The disinfectant agent is not always an electrolyte. Colloidal metals are powerful disinfectants. It has been shown that a 1 in 80,000 solution of colloidal silver sterilizes pneumococci; and about equal results have been obtained for this reagent with *B. typhosus*, *B. coli*, and dysentery bacilli.

Charrin has shown that of two lots of white mice inoculated with pneumococci, one, treated with isotonic colloidal silver of small grains, survived infection; whilst the other, retained as a control, died in thirty hours. Colloidal silver, according to this observer, is a much more powerful bactericide than salts of mercury, and is relatively non-toxic. Colloidal mercury has been shown to possess a greater germicidal power than mercuric chloride. In these cases ionization has no part.

The last phase in the disinfectant action of certain bodies (Cl, ozone, etc.) is an oxidation of living protoplasm, which, in some cases, may proceed to complete combustion. In many instances we cannot trace the action further than a precipitation of the protoplasm of the bacterial cell.

Traces of disinfectants are frequently effective; one cannot but connect this fact with another—viz., that in studying adsorption curves we see the partition between adsorbent and solvent take place in such manner, that with minimum concentrations the dissolved substance is almost completely adsorbed. As Rochaix points out, this explains why an internal antiseptic acts in the tissues despite the great dilution produced by their fluids.

Mere inhibition of development is not clearly explicable unless we invoke the intervention of adsorption phenomena.

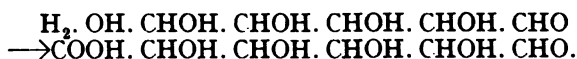
From experimental work done, it is now justifiable to conclude that in the process of disinfection one or more of three types of activity may be engaged: (1) Ionization with diffusion; (2) adsorption; (3) purely chemical action. Further, it may be concluded that the last type is usually preceded by the first and second in case the disinfectant is an electrolyte, and by the second when the

disinfectant is a colloid, and that the preliminary activities are necessary to the final action.

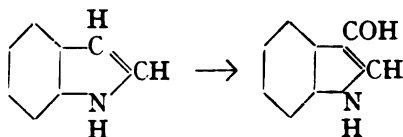
In organic compounds oxygen causes in general an increase in velocity of reaction, and tends to overcome the inertia of carbon. The linkage of carbon to carbon is loosened by the presence of oxygen, as specially seen in the fact that all carbon chains in combustion in oxygen break up into unlinked carbon dioxide. Special explosive linkages are $C\equiv C$, $O-O$, and $O-Cl$.

Hydrogen peroxide as an oxidizing agent is interesting in that its mode of action appears to be very similar to that obtaining in a number of auto-oxidations occurring in the living body. Traube conceives that in auto-oxidations super-oxides are formed by the action of oxygen carriers on molecular oxygen, and that ionization of molecular oxygen does not necessarily take place as asserted by Schönbein.

Normally saturated fatty acids in the body undergo oxidation in the β position: butyric acid becomes aceto-acetic. H_2O_2 produces the same change: $CH_3. CH_2. CH_2. COOH \rightarrow CH_3. CO. CH_2. COOH$. Glucose is oxidized in the tissues to glycuronic acid: H_2O_2 effects the same reaction—



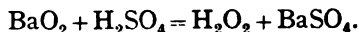
Indol is oxidized to indoxyl: H_2O_2 brings about the same reaction—



And so with other reactions.

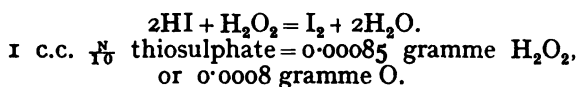
Such similarity of action is not only interesting from an academic point of view, but also from the practical, as when a mild antiseptic for use in the human subject is to be selected.

Hydrogen peroxide is prepared by acting on a peroxide of an alkaline earth by an acid, and other means—



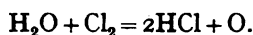
Its action as a disinfectant is somewhat slow. It is said, however, not to have the same tendency to oxidize dead organic matter as permanganates, whilst it destroys associated bacteria. It has been used to sterilize water and milk.

Estimation of H_2O_2 .—This body is sold as containing 5, 10, or 20 volumes O in solution. To 10 c.c. peroxide solution under test add about 30 c.c. H_2SO_4 (1 in 3) in a beaker (the sulphuric acid must be in fairly large excess), and crystals of KI in excess, and, after standing for five minutes, titrate the liberated I with $\frac{N}{10}$ thiosulphate and starch. Before testing, solutions of peroxide should be diluted to the strength of two volumes O—



Ozone is formed from atmospheric oxygen in a variety of ways: When phosphorus is left in contact with air, it is slowly oxidized and ozone formed. Platinum may be used for its production. Permanganates treated with concentrated H_2SO_4 yield ozone. The most common and most inexpensive method of procuring it is by means of the silent electric discharge. Electrical ozonizers have been erected in recent years for the sterilization of the water of the Marne, outside Paris, and the results have been reported as good. Various schemes have from time to time been initiated in different countries for the purification of the air of towns, public buildings, and private dwellings, by ozone; but whether advantageous results have accrued from any of these undertakings is highly doubtful.

Free chlorine is capable of killing bacteria by combining with and coagulating their protoplasm. Chlorine destroys the offensive odour of H_2S , a product of nitrogenous putrefaction, by decomposing it, with formation of HCl and $S-H_2S + Cl_2 = 2HCl + S$. But chlorine acts as a germicide for the most part, by combining with the hydrogen of water and liberating nascent oxygen—



The liberated O is the disinfectant. Light increases this reaction. The application of dry chlorine gas in disinfection may be regarded as useless.

In the so-called **chloride of lime** (a mixture of CaCl_2 and $\text{Ca}(\text{OCl})_2$) and other hypochlorites, such as chloros, Hermite solution, etc., this halogen is used in considerable quantities. Its action in all these cases is that of an oxidizer.

Chloride of lime, or bleaching powder, is produced by passing chlorine over moist lime, and is preferred to the soda and potash compounds in that it can be kept as a dry powder. The hypochlorite portion is strongly alkaline, and in the presence of moisture reacts with the CO_2 of the air to form hypochlorous acid and calcium carbonate—



In the act of disinfection, the HClO splits into HCl and nascent O . One part of fresh bleaching powder to ten parts of water has been recommended as a disinfectant solution for general work, and 1 part to 100 of water as a solution for the hands.

When solutions of chlorides of the alkalis or alkaline earths are electrolyzed, hypochlorous acid and the corresponding hydrate are formed—



Hermite applied this preparation to sanitation.

Chlorine and hypochlorites fail as disinfectants when used for materials rich in dead organic matter. Whilst the dead matter is being oxidized, the germs escape.

Estimation of Cl in Bleaching Powder.—Prepare a decinormal solution of sodium thiosulphate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. Dissolve 24.827 grammes of the crystals in a litre of H_2O .

Weigh a gramme of bleaching powder, and grind it thoroughly in a mortar. Add small quantities of water at a time, and rub into a smooth cream. Decant the liquor into a litre flask. Continue to grind the sediment with successive quantities of water until the whole is transferred to the litre flask as a fine emulsion. Make up to the mark.

Take 20 c.c. of the uniform emulsion in a basin; add excess of KI solution, dilute slightly, and acidify with acetic acid. Titrate the liberated I with $\frac{N}{10}$ thiosulphate and starch.

One c.c. $\frac{N}{10}$ thiosulphate = 0.0035.4 gramme Cl .

Another method: Prepare $\frac{N}{10}$ I, and $\frac{N}{10}$ solution of alkaline arsenite.

Mix commercial resublimed iodine with half its weight KI, and dissolve in half its weight of water. Precipitate the I with water, and filter through asbestos; wash well to remove KI, and dry over H_2SO_4 . Sublime between two large watch-glasses twice, and finally weigh out 12.7 grammes. Dissolve this in 18 grammes KI (pure) and about 250 c.c. H_2O . Make up to a litre.

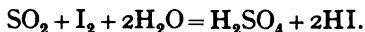
Dissolve 4.95 grammes pure sublimed and powdered As_2O_3 with 20 grammes pure sodium carbonate in about 250 c.c. H_2O . Warm and shake occasionally until solution is complete; cool and make up to a litre.

Take 20 c.c. of the well-shaken turbid emulsion of bleaching powder in a basin, and run in from a burette $\frac{N}{10}$ arsenious solution in slight excess (a drop fails to produce a blue stain on KI—starch paper). Add some starch and run in $\frac{N}{10}$ I from another burette until a slight blue colour remains. The number of c.c. $\frac{N}{10}$ I required gives the number of c.c. of arsenious solution that have been added in excess; subtract this from the total added to obtain the number of c.c. of $\frac{N}{10}$ arsenious solution equivalent to the Cl in the bleaching powder used—

One c.c. $\frac{N}{10}$ arsenious solution = 0.00354 gramme available Cl.

These methods determine quantitatively chlorinated soda, Hermite solution, chlorine-, bromine-, and iodine-water.

Sulphur Dioxide in Solution, and in Sulphite—*Estimation in Solution*.—Weigh the solution (previously cooled to 5° C. in a freezing mixture) in a stoppered flask; introduce it into a second stoppered flask, containing excess $\frac{N}{10}$ iodine. Shake thoroughly, and estimate the unchanged iodine with $\frac{N}{10}$ thiosulphate and starch—



Each c.c. of $\frac{N}{10}$ I taking part in the reaction = 0.0032 gramme SO_2 .

Estimation in Sulphite.—Powder some sulphite finely. Weigh a small quantity in a watch-glass, and introduce it immediately into a measured excess of $\frac{N}{10}$ I in a beaker. Stir until the reaction is complete, a result only slowly obtained with insoluble sulphites—

e.g., calcium sulphite. Estimate the excess iodine. It is well to do a second determination, using only a slight excess of $\frac{N}{10}$ I.

The SO_2 is calculated as above.

Bromine acts in a similar manner to chlorine by liberating nascent oxygen. Its germicidal power in the free state has been estimated as about equal to that of chlorine, but in combination with organic radicals it is superior. If careful comparative tests be made, however, it will be found that bromine is a more energetic disinfectant than chlorine, and more energetic than can be accounted for by the amount of nascent O liberated. This fact leads to the conclusion that Br acts as a disinfectant in a manner other than by liberating oxygen.

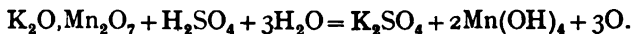
Iodine as an oxidizer is feebler than chlorine or bromine, but destroys bacteria more energetically than either by combining with their protoplasm.

Matthews found that a solution of iodine in iodide of potassium of a strength of 1 in 1,000 killed an emulsion of *Staphylococcus pyogenes aureus* in water in fifteen seconds, whilst iodoform in full dose was without action.

Permanganate of potassium, $\text{K}_2\text{O}.\text{Mn}_2\text{O}_7$, when acidified with H_2SO_4 , can yield 5 atoms of oxygen to organic matter:



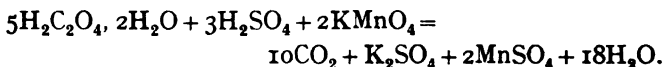
If insufficient H_2SO_4 be used, only 3 atoms of O are furnished:



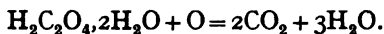
Like the other oxidizing disinfectants, its germicidal powers are expended on dead organic matter and inorganic compounds, such as sulphuretted hydrogen, ferrous salts, nitrites, etc., rather than on living bacteria. But for naked bacteria permanganates are powerful disinfectants. The disinfectant activities of oxidizers are increased by the addition of haloid acids.

Estimation of Potassium Permanganate.—Prepare $\frac{N}{10}$ oxalic acid by dissolving 6.301 grammes pure crystals in a litre of water.

On adding potassium permanganate to a warm solution of oxalic acid and sulphuric acid, the following reaction occurs:



The factors taking part in oxidation may be written more simply:



Place 50 c.c. of the $\frac{\text{N}}{10}$ oxalic acid and a little H_2SO_4 in a beaker, and dilute with water; heat to 60°C . Gradually run in from a burette the permanganate solution (about 5 grammes to the litre) until a faint permanent pink remains in the liquid after stirring. If the permanganate be added too rapidly, a brown precipitate forms, which is removed with difficulty by adding more sulphuric acid.

One c.c. $\frac{\text{N}}{10}$ oxalic acid = 0.003163 gramme potassium permanganate.

Salts of Mercury.—Of metallic salts, perchloride of mercury has had, perhaps, a larger application as a disinfectant in medicine and surgery than all the others put together. The metallic ion in solution unites with the protoplasm of the germ, causing its death. The complex appears to be of the nature of a precipitate rather than a coagulum, as it redissolves in excess of albumin. It is, therefore, necessary to use perchloride of mercury in excess. The salt is highly poisonous. The readiness with which protoplasm is precipitated by its forming albuminate and other protein compounds of mercury militates against it as a disinfectant for sputum rich in albuminoid matters, or for abscess cavities. The precipitated coat of albumin protects the enclosed bacteria from further action; hence the germs can survive, and on breaking down of the pellicle may migrate and set up infection at a distance.

The cyanide and iodide of mercury are both highly germicidal and highly poisonous. Mercury salts interfere with the action of soap.

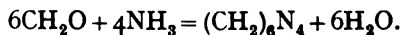
Mercuric chloride is estimated by dissolving it in HCl , and precipitating the sulphide by passing H_2S to saturation. The precipitate is allowed to stand for a time, then thrown on a filter and washed, until the washings leave no residue on evaporation. It is then dried at 100°C . and weighed. Hg is calculated from the weight of the HgS . The precipitate may contain free S , in which case it is washed with recently distilled CS_2 .

Formaldehyde, $\begin{matrix} \text{H} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{H} \end{matrix} \text{O}$, is obtained by oxidizing the vapour of methyl alcohol in the air in contact with heated platinum or

copper, and receiving the products in water. The formalin of commerce is a 40 per cent. solution of the aldehyde in water and methyl alcohol. On evaporating this solution *in vacuo* in the presence of a small amount of H_2SO_4 , a crystalline white powder falls out, of undetermined molecular weight $(\text{CH}_2\text{O})_n$, and known by the names 'paraformaldehyde' and 'paraform.' This polymer is volatilized on heating into formaldehyde. Both the liquid and solid forms are used in disinfection. An enormous amount of work has been done on the properties of formaldehyde as a germicide, and everyone is agreed that as such it holds a high position. For application to rooms the solution may be heated, or the solid may be volatilized over a lamp. There can be little doubt that the interaction between formaldehyde and the protoplasm of the germ is of the nature of a coagulation. Its powerful reducing properties remove oxygen from the protoplasm, probably both from hydroxyl groups and from the oxygen united directly to carbon.

It is used for the floors, walls and ceilings of rooms as a spray, in the form of vapour produced by an autoclave under pressure, and as the vapour of paraform produced by a lamp. For spray work various strengths of solution have been recommended, ranging from 0.5 per cent. to 2.5 per cent. and higher. Some suggest supplementing the spray with vapour, more especially where rooms are exceptionally dirty, and unknown organisms like that of smallpox are being dealt with. In the present state of practical disinfection a wide margin of safety should be insisted on. It is possible that in some circumstances the highest concentration recommended fails to sterilize.

Estimation of Formaldehyde.—Formaldehyde slowly absorbs ammonia to form hexamethylene-tetramine; 180 parts formaldehyde react with 68 of ammonia:



Place 10 c.c. of the solution to be tested in a flask, and neutralize, if necessary, with $\frac{N}{100}$ NaOH; dilute with water, and treat with an excess of standard ammonia solution. It is well to stand overnight. Distil the excess of ammonia by a current of steam into standard acid. Calculate the percentage amount of formaldehyde from the amount of ammonia combined.

The success which attended the early application of Carbolic Acid as an antiseptic by Pasteur, Lister, and others, attracted attention to coal tars as a source of germicides.

By suitable fractional distillation these tars can be separated into—(1) First runnings; (2) light oils; (3) heavy oils; (4) anthracene oils.

Carbolic acid is contained for the most part in the light oil fraction; whereas the heavy oil fraction contains its homologues, especially the cresols.

At first acid and alkaline solutions of crude carbolic acid were used as disinfectants, but it was soon found that these were not suitable. Pure watery solutions of cresols were then tried, and likewise abandoned for saponified emulsions. It was discovered that emulsions conferred increased germicidal efficiency on the various active phenolic bodies used, and that side-chain substitution in the benzene ring produced the same result.

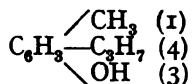
It was also discovered that metacresol, the least soluble in water, had a higher germicidal power in emulsion than ortho- or paracresol.

The relative solubilities of the three isomers in water are—

| | | | | | Per Cent. |
|-------------|----|----|----|----|-----------|
| Orthocresol | .. | .. | .. | .. | 2.5 |
| Metacresol | .. | .. | .. | .. | 0.53 |
| Paracresol | .. | .. | .. | .. | 1.8 |

Two important stages in the evolution of coal-tar disinfectants had now been reached and passed. The emulsion was better than the solution: insolubility in water was of advantage in the same direction.

The high germicidal properties of thymol illustrate these principles, containing as it does three side-chains attached to the benzene ring:

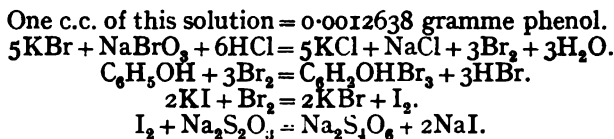


Its molecular weight is much higher than that of phenol ($\text{C}_6\text{H}_5\text{OH}$). Its solubility in water is about 1 in 1,100, as against 1 in 15 for phenol. Koch found that the same germicidal work was performed

on anthrax bacilli by thymol in dilution of 1 in 80,000 as by phenol in 1 in 1,250.

Estimation of Phenols.—The following method is based on the precipitation of phenol from its aqueous or alcoholic solution by bromine as tribromphenol.

Prepare a standard solution consisting of 2.04 grammes sodium bromate and 8.00 grammes potassium bromide in a litre of water.



Weigh out a gramme or two of the phenol to be tested in a tared watch-glass, and dissolve in excess of NaOH. Make up to, say, 500 c.c. Take 20 c.c. (one-twenty-fifth of the whole) in a 300 c.c. stoppered flask, and add 25 c.c. of the standard bromide bromate solution. In a second 300 c.c. stoppered flask place 25 c.c. of the standard bromide bromate solution.

To each add 5 c.c. pure HCl and shake. Add such a further measured quantity of the standard bromide bromate solution to the phenol flask that, on shaking, the white tribromphenol is left distinctly yellow (excess Br). Shake well and stand for fifteen minutes. Add excess KI to both flasks, and titrate with a solution of thio-sulphate of Na (say 10 grammes to a litre).

Example.—2.168 grammes phenol required 75 c.c. standard bromide bromate to become yellow. The iodine which the free bromine liberated required 16.8 c.c. thiosulphate. But 25 c.c. bromide bromate in second flask required 51.8 c.c. thiosulphate. Therefore 16.8 c.c. thiosulphate = 8.18 c.c. bromide bromate solution. Therefore $75 - 8.18 = 66.82$ c.c. bromide bromate solution which interacted with phenol. Therefore $66.82 \times 0.0012638 \times 25 = 2.111$ grammes phenol.

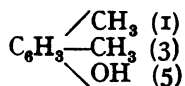
$$2.168 : 2.111 :: 100 : 97.4.$$

That is, this sample contains 97.4 per cent. pure phenol.

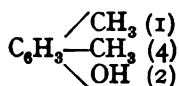
Laubenheimer showed that a 1 per cent. solution of phenol required ninety minutes to kill a quantity of staphylococci, whereas

the same strength of a solution of propyl-phenol, $\text{C}_6\text{H}_4\begin{smallmatrix} \diagup \text{C}_3\text{H}_7 \\ \diagdown \text{OH} \end{smallmatrix}$, did the same work in three minutes.

Increase in molecular weight does not always mean increase in germicidal power; because, in the same series of Laubenheimer's experiments, isopropyl-phenol required twelve minutes to kill. Working with *m*-xylenol,

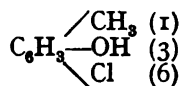


symmetric, and *p*-xylenol,

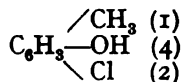


he found that the meta-compound was much more powerful than the para- in killing staphylococci.

On incorporating an atom of chlorine in meta- and para-cresols, he found that chlor-*m*-cresol,



still retained its advantage, and even increased this advantage over chlor-*p*-cresol,



The relative position of the side-chains in the ring is thus shown to be of importance. It may very well be that the disinfection process is assisted by the meeting of suitable side-chain affinities in microbe and disinfectant.

Decrease in solubility in water means decrease in toxicity. It is possible, therefore, to apply to the skin and intestinal mucosa insoluble phenyloids in concentrations which could not be tolerated in phenol.

Henle, working twenty years before Laubenheimer, showed that the germicidal powers of the cresols varied with their boiling-points, the meta-compound, with highest boiling-point, possessing

the most intense action, and the ortho-, with the lowest boiling-point, the least intense action.

Working with higher phenols, Sommerville found that the same principle obtains. Using the Rideal-Walker method of estimating germicidal efficiency, and emulsionizing fractions from the same distillate of blast-furnace phenyloids, he found that a fraction boiling at 248° possessed a coefficient three points above that of another fraction boiling at 220° , and five points above that of a third fraction boiling at 207° .

Again, it is possible to alter by several points the coefficient of a phenyloid by varying the chemical or physical characters of the emulsion.

Changes which make for increased adsorption raise (within limits) the coefficient. Increased viscosity in the emulsion lowers (within limits) the coefficient.

If a liquid is contained between two parallel plates, and one of these be moved with a constant velocity in its own plane, a certain force is required which depends on the velocity, the surface, and distance, of the two plates, and on the temperature and nature of the liquid. The force required to move a plate of unit surface separated from another plate of the same size by a layer of liquid of unit thickness at unit velocity is known as the viscosity coefficient.

Colloidal solutions may be divided into two classes if the increase of viscosity compared with that of the continuous phase (solvent) be made the basis of classification. One class presents a viscosity only slightly higher than that of water (metal and sulphide solutions). The other, the organic colloids (albumin, gelatin) presents a marked increase of viscosity. In those solutions presenting a low viscosity, the disperse phase is present as solid particles; in those with high viscosity, the disperse phase is liquid. Albumin solutions consist of a dilute solution of albumin, in which are dispersed globules of a more concentrated solution. Systems of solid particles of microscopic size distributed in a liquid are known as 'suspensions'; those consisting of two liquid phases are known as 'emulsions.'

The particles in a solution, if sufficiently small, are in constant motion, oscillating round a central position, and also undergoing an irregular translatory motion. Svedberg showed that the ampli-

tude of the motion of a particle is directly proportional to the period, and inversely proportional to the viscosity, of the liquid. Perrin showed that this Brownian movement conformed to the principles of the kinetic theory, and that the particles could be treated as large molecules. The stability of the solution is intimately connected with the electric charge. The charge can be altered by the addition of electrolytes, and may fall to zero with suitable concentrations, in which last case the solutions precipitate. It has been long known that the speed of settling of such suspensions can be increased by the addition of electrolytes.

In systems of two liquid phases, it can be shown that very small liquid particles approaching ultramicroscopic dimensions possess a high degree of rigidity. Systems of two liquid phases possessing few and widely separated particles differ in no important respect from systems containing rigid particles; but an important difference appears as the amount of disperse phase per unit volume increases. In the case of rigid spherical particles in contact, the disperse phase may reach a maximum of 74 per cent. of the total volume. If the disperse phase be liquid, the globules may not merely touch one another, but become flattened at the points of contact, from which circumstance it is obvious that there is no limit to the ratio $\frac{\text{vol. of disperse phase}}{\text{total vol.}}$, which ratio may approach unity. It is

not possible to prepare emulsions containing such percentages of disperse phase unless the continuous phase is a solution of certain substances, such as soap. Such bodies froth, an indication that the dissolved substance lowers the surface tension of the solvent. The process of emulsification is intimately connected with such lowering of surface tension, or, rather, interfacial tension between the two phases.

The stability of emulsions varies considerably. They are destroyed by the addition of all substances which destroy the emulsifying agent; thus, emulsions made with soap solution are destroyed by the addition of an acid which decomposes the soap.

In the making of an emulsion, the two phases are shaken up until the disperse phase is sufficiently finely distributed. In the case of gelatin emulsions and soap emulsions, the behaviour of the solution is not to be explained unless by assuming that it is a system of two

fluid phases; in other words, it consists of globules having a high gelatin content in a continuous phase which is a dilute solution of gelatin. The solvent here may be shifted most readily from one phase to the other. Different behaviour is shown by the albumins. Egg albumin is soluble in water, and does not form a gel, either by cooling or concentration, but it coagulates irreversibly at a temperature of about 60°C . The temperature of coagulation can be changed by adding salts, and may be raised to over 100° by the addition of a thiocyanate. In relation to this phenomenon is the change which follows the addition of alkali salts in the cold—the coagulation known as ‘salting out.’

If at the boundary surface between the phases of a disperse system a change in the concentration of either phase will lead to a decrease of surface tension, this change will occur. The change in concentration is adsorption. It requires work to make or enlarge a surface; when such surface is made, it is the seat of energy. As we have seen above, adsorption plays probably an important rôle in disinfection. Soap emulsions of coal-tar phenyloids can be constructed which are eminently suitable for the production of this phenomenon. Such emulsions when compared with suspensions show a decreased size of particle with reduced velocity of settlement, increased Brownian movement with increased electric charge, due to the great increase of specific surface. These emulsions provide for a high degree of bombardment of the microbe by the active particles of disinfectant, followed by marked adsorption, both necessary preliminaries to the final chemical action required to kill the organism.

In most of the modern better-class disinfectants distilled from tar, and emulsionized in soaps, the active principles are phenyloids. In the raw materials these bodies are mixed with neutral oils, saturated paraffins, unsaturated paraffins (olefines, etc.), pyridines, and a mass of heterogeneous substances.

The unsaturated hydrocarbons are washed out with H_2SO_4 , and the phenyloids with NaOH (formation of sodium phenylates). Separation is made in laboratory practice in separator funnels. The addition of a few drops of alcohol sometimes assists the separation.

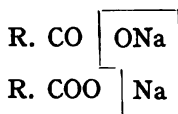
Sodium phenylates are split with H_2SO_4 , and the free phenyloids

recovered. These may be fractionally distilled, and the fractions emulsioned. Hard waters, including sea-water, 'salt out' soap emulsions in varying degrees. Such waters should be softened before using them for diluting soap emulsions of phenyloids. Gelatin or glue, whilst not forming so good an emulsion as soaps, is not attacked by hard waters to the same degree.

To determine the percentage composition of a coal-tar disinfectant in a soap emulsion, fractionally distil 100 grammes of the disinfectant. Measure the water and weigh the phenyloids. Below 270°C . resin gives no trouble, as any resin spirit present (never more than 5 per cent. in resin soap) is in union with alkali, and resin oils boil between 300° and 400°C . Should a small quantity of neutral oil come over, which rarely happens, it may be separated with the phenolic bodies by washing with soda, and subsequently splitting off the phenyloids with H_2SO_4 .

Five grammes of the disinfectant are incinerated, resulting in Na_2CO_3 or K_2CO_3 . The residue is lixiviated with water, filtered, titrated with standard HCl , and calculated as Na_2O or K_2O . The weight of the chloride will at once determine whether one is dealing with K or Na .

As the residue in the distillation retort consists of anhydrides of fatty acids or resin acids, or of both, of the form



it is plain that in the original disinfectant those anhydrides plus H_2O are equivalent to the Na_2O . Hence 5 grammes disinfectant minus weight of Na_2O equals fatty acids plus resin in 5 grammes. If the fatty acid and resin figures are required separately, they can be easily worked out from the retort residue by Twitchell's method.

In 1903 Rideal and Walker published a method of standardizing disinfectants. The method has since undergone slight modifications, and to-day is carried out as follows: The materials required for the test are a standard nutrient bouillon, standard carbolic acid, dilution of the disinfectant, and the broth culture. The nutrient bouillon is composed of 20 grammes of Liebig's extract of

meat, 20 grammes of Witte's peptone, 10 grammes of sodium chloride, and 1 litre of distilled water. This mixture is boiled for thirty minutes, filtered, and neutralized with normal sodium hydrate, using phenolphthalein as indicator. To avoid contaminating the broth with phenolphthalein, a small aliquot part, say 10 c.c., should be taken out and titrated with $\frac{N}{10}$ NaOH; from the result obtained a calculation is made of the amount of normal sodium hydrate necessary for the neutralization of the remainder of the broth. When quite neutral, 15 c.c. of N.HCl is added. The broth is then made up to a litre and sterilized. Where 2 or 3 litres are prepared at one time, as is customary, the broth is distributed in 500 c.c. flasks on the following day and again sterilized. With the aid of a small separating funnel, 5 c.c. are then run into sterile test-tubes, which, after plugging with sterile cotton-wool, are placed in the steam sterilizer for half an hour.

As carbolic acid crystals are frequently contaminated by cresols to such an extent as to make them unreliable for purposes of bacteriological control, their purity should be established by a determination of the solidifying-point on at least 50 c.c. of material with the thermometer in the liquid. The point is very sharp, the thermometer showing a constant temperature for a period of from five to ten minutes. The solidifying-point of the crystals is 40.5, but anything over 40 may be accepted. A 50 per cent. by weight stock solution is then prepared and standardized by titration with decinormal bromine. From this solution, which keeps indefinitely in stoppered bottles, the various working strengths are made by diluting a comparatively large quantity, such as 100 c.c., to the desired volume; this serves to eliminate the error introduced by measuring out small quantities of strong acid.

In preparing dilutions of the disinfectant, a stock solution or emulsion should be prepared in a 100 c.c. stoppered cylinder with sterilized distilled water—10 per cent. if the coefficient be under 1, and 1 per cent. if over 1. Ten c.c. of this stock are used in preparing each of the four dilutions required for the test. Thus, working with a sample having a coefficient under 1, if it is desired to prepare a dilution 1 in 70, 10 c.c. of the 10 per cent. stock solution are diluted with 60 c.c. of distilled water; and in the case of a preparation having a coefficient over 1, where the dilution required is 1 in 700, 10 c.c.

of the 1 per cent. stock solution should be diluted with 60 c.c. water.

The culture of *B. typhosus* is incubated for twenty-four hours at 37° C. in Rideal-Walker broth. It is advisable to make a subculture every twenty-four hours from the previous twenty-four-hour culture, even if on many days no test is performed; but, as this tends to attenuate the organism, it should be continued for not longer than one month, when a fresh subculture in broth should be taken from an agar culture one month old. This procedure secures a test culture varying but little from day to day in resistance offered to disinfectants, and renders the selection of the appropriate dilution of carbolic acid easier than if the culture from which the twenty-four-hour growth is obtained were older on one occasion than on another.

The apparatus required consists of a test-tube rack, an inoculating needle, test-tubes, and a dropping pipette. The test-tube rack possesses two tiers, the upper having holes for thirty test-tubes in two rows, each row containing three sets of five. The upper tier holds sterilized broth tubes, each of which is numbered with a grease pencil. The lower tier holds the medication-tubes, four containing the postulant disinfectant dilutions, and one the carbolic acid control dilution. This tier is provided with a copper water-bath intended to preserve the temperature of medication within the prescribed limits (15° C. to 18° C.). The test-tubes are numbered in rotation; and it will be seen that the first medication-tube is used for inoculating broth-tubes—1, 6, 11, 16, 21, and 26; the second for inoculating, 2, 7, 12, 17, 22, and 27, etc.

The needle recommended is a thin aluminium rod carrying a short piece of platinum wire, 0.018 inch in diameter (26 U.S. gauge), passed through and twisted round an eye in the end of the rod. A loop 3 millimetres internal diameter is formed on the end of the wire. The length of the wire to the end of the loop should be about 1½ inches. A fairly uniform drop can be obtained after a little practice by dipping the needle in the medicated culture, and bringing it out with a slight jerk.

The test-tubes should be of strong glass, so as to minimize the risk of breakage, and lipped to facilitate the manipulation of plugs. The size recommended is 5 inches by ⅝ inch.

The cotton-wool plugs for both medication-tubes and broth-tubes

should be well made, so that they can be withdrawn and replaced without loss of time.

The dropping pipette is standardized to deliver 0.1 c.c. of the broth culture per drop. It is loosely plugged at the top with cotton-wool, and when not in actual use is kept in a sterile test-tube plugged at the mouth with cotton-wool. For greater convenience, the tube should be passed through the centre of the plug, and fastened thereto with wire. In addition to these, one or two of each of the following are required: 1, 5, and 10 c.c. pipettes; 100 and 250 c.c. stoppered cylinders, with inverted beakers, to safeguard against dust after removal from sterilizer; wire baskets to receive tubes for incubation or sterilization. All pipettes and cylinders should be standardized.

Before commencing the test, it is necessary to ascertain the carbolic acid control dilution which will give the desired result—*i.e.*, life in two and a half and five minutes. This is done by running a trial test with five dilutions of the carbolic acid only—say 1 in 80, 1 in 90, 1 in 100, 1 in 110, and 1 in 120. Five c.c. of the control solution so ascertained are then pipetted into the fifth medication-tube, the other four receiving 5 c.c. of the various dilutions of the disinfectant under test. To save time and apparatus, one pipette can be made to do service at this stage by starting with the phenol solution, and following on with the highest or lowest dilution of the disinfectant, according as the coefficient is below or above 1, rinsing out the pipette in each case with the next dilution before measuring off the sample for test.

The plug of the culture-tube is now replaced by the culture pipette, which, as explained above, has a plug attached to it with wire, at such a height that, when the plug fits easily into the mouth of the culture-tube, the point of the pipette is halfway down the broth, and clear of the clumps. The first of the five medication-tubes is now inoculated with five drops of the culture—*i.e.*, 0.5 c.c. At intervals of half a minute each of the other medication-tubes is inoculated in turn. By the time the fifth tube has been inoculated, the organism in the first will have been exposed to the action of the disinfectant for two minutes, and after the next half-minute a loopful of the latter is inoculated into the first broth-tube, loopful from the other medication-tubes being in turn inoculated into their respective broth-tubes at the rate of one every thirty seconds. By

the time the fifth broth-tube has been inoculated from the fifth medication-tube, the disinfectant in the first medication-tube will have acted on the test organism for four and a half minutes, and after the next thirty seconds a loopful is introduced into broth-tube 6, and so on. The actual test, therefore, occupies seventeen minutes, and provides for six two-and-a-half-minute periods of contact in each of the five medication-tubes.

It is open to the worker to adopt any convenient method of manipulating the tubes and plugs. The following procedure is given for the guidance of the inexperienced: The first medication-tube is taken from the rack, and the contents gently agitated for a second to insure even distribution of the bacilli; the plug having been taken out and grasped by the left little finger, the tube is held between the back of the left forefinger and front of the second. The corresponding broth-tube (No. 1) is taken up by the right hand and transferred to the left between the thumb and forefinger, the plug being extracted and held by the little finger of the right hand. The tubes now being in position for inoculation, the needle, which should have been sterilized before the tubes were touched, is introduced into the medication-tube, from which a loopful is taken and inoculated into the broth-tube. The needle is sterilized in the flame (placed to the right), and pushed with a movement of the thumb well up between the first and second fingers of the right hand; the plugs are then replaced, the medication-tube going back to the rack, while the broth-tube is subjected to a gentle agitation and placed in a wire basket on the right of the rack. This basket, containing the thirty inoculation-tubes and test form, giving particulars of the dilutions, etc., is now placed in the incubator, where it is allowed to remain for forty-eight hours at blood heat, when the results are read off. A moment's consideration of the manner in which the test has been conducted will suffice to indicate where the results of each subculture should be placed in the table.

The following details of a test of a disinfectant marked 'A' show the form in which the results are set out; incidentally it shows the degree of refinement to which the test can be carried with a little practice and care.

The strength or efficiency of the disinfectant under test is expressed in multiples of carbolic acid, and is obtained by dividing

the dilution of the disinfectant showing life in two and a half and five minutes by the carbolic acid dilution, which of course must show the same result. In the present instance this 'figure of merit,' or Rideal-Walker coefficient, is 16.6.

To avoid annoyance and loss of time caused by aerial contamination of tubes, etc., it is advisable to conduct the test in a room free from draughts; a further safeguard is provided by spraying or swabbing the floors and benches with an efficient disinfectant solution. Needless to add, all pipettes, etc., must be rigorously sterilized before use.

In this, as in all other arbitrary tests, the need for strict observations of the conditions of the test is imperative.

B. TYPHOSUS: TWENTY-FOUR HOURS' BROTH CULTURE AT 37° C.

Temperature of medication 15° C. to 18° C.

| Sample. | Dilutions. | Time Culture exposed to Action of Disinfectants (Minutes). | | | | | | Subcultures. | |
|---------------|------------|--|----|-----|-----|------|-----|-----------------------|--------------|
| | | 2½. | 5. | 7½. | 10. | 12½. | 15. | Period of Incubation. | Temperature. |
| A | 1 : 1,900 | x | — | — | — | — | — | 48 hours | 37° C. |
| " | 1 : 2,000 | x | x | — | — | — | — | " | " |
| " | 1 : 2,100 | x | x | x | — | — | — | " | " |
| " | 1 : 2,200 | x | x | x | x | — | — | " | " |
| Carbolic acid | 1 : 120 | x | x | — | — | — | — | " | " |

$$\therefore \text{Rideal-Walker coefficient } \frac{2,000}{120} = 16.6.$$

APPENDIX

FLOCK manufactured from rags, to be used in upholstery, bedding, etc., must meet the standard of cleanliness laid down by the Local Government Board's Rag Flock Regulations, 1912—viz., not to contain more than 30 parts chlorine per 100,000 parts flock, the chlorine to be removed as chlorides with distilled water at a temperature not exceeding 25° C. from not less than 40 grammes of a well-mixed sample of flock.

Steep 50 grammes of a mixed sample of flock in $\frac{1}{2}$ litre of distilled water overnight. Decant the fluid on a filter, and squeeze out the flock thoroughly. Wash the flock with smaller quantities of water (say 100 c.c.) three or four times, squeezing out all the water possible each time, and passing the washings through the same filter. Make up the filtrate to a litre. Now evaporate 100 c.c. of this (= 5 grammes flock) to dryness with a small quantity of CaO in a platinum dish, and char the residue. When cool, extract with 50 to 100 c.c. distilled water and filter. Add a few drops of potassium chromate to the filtrate, and run in from a burette silver nitrate solution (used in estimation of Cl in water), 1 c.c. of which equals 1 milligramme Cl. Multiply the number of c.c. used by 20 to obtain parts Cl per 100,000 flock.

Copper Sulphate is used for greening peas and other vegetables:

Estimation of Copper.—Ash 10 grammes of the peas or other material. Moisten the ash with concentrated HNO_3 ; add water, and boil. Make strongly alkaline with ammonia, and filter. If no blue colour, copper is absent. If blue, transfer the fluid to a Nessler glass on a white tile, and match the colour against weighed small quantities of copper sulphate converted into ammoniacal solution in the same manner.

Or, the copper may be deposited in the metallic state by passing an electric current through the acid solution, in a suitable apparatus, and weighed as Cu.

Tin in Canned Food.—See Local Government Board Reports of Inspector of Foods, No. 7; Report of Buchanan and Schryver.

1. *Colorimetric Method*.—Prepare a solution of stannous chloride containing 0.286 gramme per 100 c.c.

Prepare a solution of dinitrodiphenylaminesulphoxide containing 0.2 gramme in 100 c.c. $\frac{N}{10}$ NaOH. Mix 10 parts HNO_3 (sp. gr. 1.48) with 10 parts HNO_3 (sp. gr. 1.4). Cool this mixture with ice, and add 1 part of thiodiphenylamine (prepared by heating diphenylamine with sulphur) in small quantities at a time with constant stirring. Do not allow the temperature to rise above 5°C ., and add such small quantities at a time that a hissing sound is hardly perceptible when the solid comes into contact with the liquid mixture. The thiodiphenylamine dissolves at the beginning to form a clear solution of red colour, which, before the whole of the amine has been added, commences to thicken, owing to the separation of the nitro-body. After standing for some hours (not more than half a day), suck off the nitro-body on an asbestos filter, and wash first with concentrated HNO_3 , then with acid of gradually diminished strength, and finally with pure water. Now extract it with hot alcohol in which it is not appreciably soluble.

Introduce 10 grammes of the food into a 700 c.c. Kjeldahl flask; add 10 grammes of potassium sulphate and 10 c.c. concentrated sulphuric acid. Heat over small flame till mixture chars and froths. Add another 10 c.c. H_2SO_4 , and regulate the size of the flame so that the H_2SO_4 can be boiled without loss from frothing. Heat till the contents of the flask are quite white. Cool; dilute with water to about 100 c.c. Pass in H_2S gas, and let stand in a corked flask overnight. Warm slightly on a water-bath, and filter off the precipitated sulphide and sulphur. Transfer the filter-paper containing the precipitate to a test-tube, and boil with 5 c.c. concentrated HCl to dissolve the sulphide. Filter through a small conical Buchner funnel into a wide-mouthed test-tube, with a side-tube near the top to connect with a pump. Suck as dry as possible, and wash with 2.5 c.c. concentrated HCl . Connect the wide-mouthed test-tube with a CO_2 generating apparatus, and pass the gas through a tube which passes through a cork inserted in the mouth of the test-tube, and which reaches nearly to the surface of the liquid. The side-tube serves as an exit for the gas. Whilst still hot, throw into the strongly acid liquid a strip of zinc foil 2 inches long, 0.5 inch wide, and weighing about 0.75 gramme, and the stannic chloride is reduced to stannous chloride. As soon as the last traces of Zn are dissolved, add 2 c.c. of the reagent by pipette to the hot liquid, the CO_2 passing the while. On addition of the reagent, the nitro-body is precipitated. On warming, it passes again into solution in the concentrated acid. Boil the solution for a minute or two, and dilute to 100 c.c. with cold water. Filter the dilute solution by means of a pump from the unchanged nitro-body. The solution usually turns violet during filtration; the full depth of colour is rapidly attained

by addition of a drop of dilute ferric chloride. It is then matched with known quantities of the standard tin solution.

2. *Gravimetric Estimation.*—Fifty grammes of the food are incinerated in two lots of 25 grammes in two Kjeldahl flasks of about 700 c.c. capacity, using 25 c.c. of H_2SO_4 previously diluted with 100 c.c. water, and 25 grammes potassium sulphate. When thoroughly charred, another 25 c.c. concentrated H_2SO_4 are added, and heat continued till contents are white (perhaps four to five hours required). The contents of the two flasks are brought together and diluted to about 600 c.c. H_2S gas is passed, and the mixture allowed to stand corked overnight. It is next warmed, and the mixture of sulphide and sulphur filtered through a small filter-paper 7 centimetres in diameter. The precipitate is washed on the filter-paper with warm water. With it are usually mixed bodies other than sulphur and sulphide, such as silica derived from the flask, etc. To separate these, the sulphide is dissolved on the filter-paper in a small quantity (10 to 20 c.c.) of hot 10 per cent. NaOH . From the yellow solution obtained the sulphide is reprecipitated by glacial acetic acid, filtered off, washed with hot water, dried, oxidized, and weighed as oxide of tin.

Estimation of Organic Matter in Air.—In addition to the microscopic examination of dust and suspended matters in the air described at p. 143, it may be necessary in certain cases to estimate organic matter quantitatively. This may be roughly done thus: Aspirate a measured volume of air through a tube containing a plug of clean glass-wool, and digest the wool in standard potassium permanganate (p. 48) for an hour at 37°C . Titrate the permanganate with standard oxalic acid (0.7875 gramme crystals to a litre). Perform a blank experiment, and deduct the number of c.c. oxalic acid used from that used in the actual estimation. The result is recorded in terms of O absorbed from permanganate. 1 c.c. oxalic acid = 1 c.c. permanganate = 0.1 milligramme O.

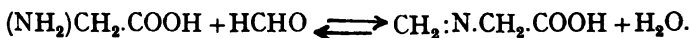
Haldane's Apparatus for Estimating CO_2 in the Air.—This consists of an air burette (enclosed in a water-jacket with a glass face) with a wide ungraduated and a narrow graduated portion. Its capacity is 20 c.c. from top to bottom of scale. The graduated portion measures 4 inches in length, and is divided into 100 equal parts, each corresponding to one-ten-thousandth part of the capacity of the burette when moist for mercury. Readings are recorded in parts per 10,000 without calculation or corrections. The water in the water-jacket is stirred up occasionally in order to secure uniformity of temperature.

In using the apparatus, the air is first expelled by a three-way tap from the burette by raising the mercury bulb attached to its lower end. Air is then taken in by lowering the bulb till the mercury falls to the zero of the graduated scale. The tap to the absorption pipette (the latter filled to a mark with 10 per cent. KOH) is next opened, and the air is driven over and drawn back several times till all CO_2 is absorbed as indicated by constant level of Hg. The difference between the first and last readings gives the amount of CO_2 in parts per 10,000.

Estimation of Formaldehyde in Meat Foods.—See Local Government Board Food Reports, No. 9. Schryver points out that in meat foods it is possible that the formaldehyde may be entirely oxidized to CO_2 and H_2O by tissue oxidases; that part of the formaldehyde may be polymerized to paraformaldehyde; and that formaldehyde may enter into chemical combination with some of the constituents of the foodstuffs. He has confirmed the statement made by Cervello and Pittini, and by Batelli and Stern, that formaldehyde is destroyed by tissue oxidases.

When formaldehyde solution is distilled, the distillate contains less aldehyde than the original solution, due to polymerization by heat into a non-volatile polymer. It is therefore not possible to estimate formaldehyde by steam distillation.

Schiff and Sørensen have shown that formaldehyde reacts with proteins and amino-acids, with formation of methylene-imino compounds, and that the reaction is a reversible one, and only proceeds to completion in presence of large excess of formaldehyde:



Amino-acids, owing to basic and acidic groups, have an amphoteric reaction, and become acid on treatment with formaldehyde: the number of amino-groups in combination can be accordingly determined by titration with alkali. Conversely, it is possible by titration to estimate the amount of formaldehyde which can enter into combination with any product. Meats contain relatively large quantities of substances which are capable of entering into chemical combination with the aldehyde. The reaction, as already mentioned, will not proceed to full completion except in presence of excess of aldehyde, owing to the reversibility.

In addition to these reversible compounds, formaldehyde can combine with proteins to form relatively stable insoluble products, from which formaldehyde can be eliminated only by prolonged heating with water.

Any effective method for estimating formaldehyde in meat must therefore be applicable to estimation of free aldehyde, the polymerized product, and aldehyde in combination with the meat.

The violet colour obtained when milk containing formaldehyde is heated with strong HCl in the presence of an oxidizing agent cannot be used to detect aldehyde in meat, as meat gives a violet colour on warming with HCl in the absence of the aldehyde due to the formation of hæmatoporphyrin from hæmoglobin.

The following method is recommended:

To 10 c.c. of solution containing aldehyde add 2 c.c. of a freshly prepared and filtered 1 per cent. solution of phenylhydrazine hydrochloride. To this add 1 c.c. of a 5 per cent. fresh potassium ferricyanide solution, and 4 c.c. of concentrated HCl. In the presence of formaldehyde a brilliant fuchsin-like colour is produced, which reaches its full intensity after a few minutes' standing, and keeps without marked deterioration for several hours.

The addition of ferricyanide oxidizes the formaldehyde condensation product to a substance which is a weak base, which forms a scarlet hydrochloride. This, on dilution, undergoes hydrolytic dissociation, yielding a base which can be extracted with ether to form a yellow solution. If this latter be shaken with concentrated HCl, the base passes back into aqueous solution in the form of the scarlet hydrochloride. This reaction detects formalin in concentration of 1 part in 1,000,000. It is quantitatively best applied when the concentration is 1 in 50,000.

From two standard solutions containing respectively, 1 in 10,000 and 1 in 100,000, it is possible to make a series of dilutions from 1 part in 1,000,000 upwards to serve as a colour scale when the reaction is quantitatively applied.

Methylene-imino derivatives can be readily hydrolysed by cold water; with ammonia, formaldehyde forms a somewhat more stable derivative; and with Witte's peptone, under certain conditions, an insoluble product from which formaldehyde is only eliminated with some difficulty.

By modification of the above reaction formaldehyde can be detected in all such combinations. If the mixture containing such product be warmed after addition of phenylhydrazine, the aldehyde after scission combines immediately with phenylhydrazine to form a stable condensation product. This reaction, being irreversible, proceeds to completion. On the addition of the ferricyanide and HCl, the colour is developed in its full brilliancy.

In the same manner, by heating after addition of phenylhydrazine, formaldehyde can be detected when present in its polymerized form.

Heat 10 grammes of meat (minced) with distilled water on a boiling-water bath for five minutes. Where the concentration is 1 part formaldehyde in 50,000 or less, 10 c.c. of water is sufficient. Where the concentrations are higher, larger quantities of water must be employed. To every 10 c.c. of water used add 2 c.c. of a 1 per cent. phenylhydrazine hydrochloride solution. Cool and filter from

the coagulum through cotton-wool. To 12 c.c. of the filtrate add 1 c.c. 5 per cent. potassium ferricyanide and 4 c.c. concentrated HCl. Compare colour with standards made from the standard formaldehyde solutions.

It has been found that in chilled beef treated by formaldehyde the superficial fat contains distinct quantities of formaldehyde; muscular tissue unprotected by fat is more largely contaminated than other parts.

Grilling of meat but slightly diminishes the amount of formaldehyde, and apparently causes the aldehyde to penetrate farther into the interior. Boiling greatly diminishes it. Roasting gets rid of most of it. Sausages made from meat impregnated with formaldehyde and cooked in the ordinary way, retain it. A common depth of penetration into muscular tissue is 20 millimetres.

Arsenic in Foods.—See Reports of the Royal Commission on Arsenical Poisoning, 1903. Cd. 1869. Minutes of Evidence and Appendices, Vol. II., especially Appendices 16, p. 183; 19, p. 201; 20, p. 206; 21, p. 208; 22, p. 220; 24, p. 230.

Estimation of Arachis Oil found as an Adulterant in Olive Oil.—Saponify 5 grammes of the sample with 25 c.c. alcoholic potash solution (8.5 per cent.). Add that quantity of acetic acid which has previously been found by titration to exactly neutralize 25 c.c. of the above alcoholic potash, and cool the vessel in water. Let stand for two hours. Filter off the acids on a filter-paper, and wash them with 70 per cent. alcohol containing 1 per cent. HCl. Dissolve the acids on the filter with about 40 c.c. boiling alcohol (95 per cent.). Add about 10 c.c. of water to bring down the alcohol to about 20 per cent., and cool down to room temperature. Filter after an hour, and wash the precipitate with 70 per cent. alcohol. Dry the precipitate (arachidic acid) at 100°, and weigh. As arachidic acid forms about 5 per cent. of arachis oil, the weight of the oil is readily calculated.

Baking-Powders.—These preparations consist of an acid and an alkaline constituent, and a third inert body—generally starch—intended to absorb moisture, and thereby prevent premature chemical action. The alkaline constituent is almost always bicarbonate of soda. The acid constituent may be (1) tartaric acid or an alkaline bitartrate; (2) acid phosphate of calcium; or (3) an alum.

Whilst sodium bicarbonate and tartaric acid are free from calcium sulphate, acid calcium phosphate (used in the manufacture of

baking-powder and self-raising flour) always contains more or less of this contamination.

Estimation of CaSO_4 .—(a) Ca: Dissolve 10 grammes of the sample in boiling dilute HCl; add slight excess of ammonia, then slight excess of acetic acid, and filter off any precipitate that may form. To the filtrate add excess of ammonium oxalate; collect the precipitate of calcium oxalate on a filter; wash; dry in an air oven; ignite; cool and weigh as CaO .

(b) Sulphate as SO_3 : Dissolve 10 grammes of the sample in boiling dilute HCl as above; add slight excess BaCl_2 , and allow the precipitate of BaSO_4 to settle. Filter; wash the precipitate free from chloride; dry in air oven; incinerate; cool and weigh. The weight of the ash minus the weight of the ash of the filter-paper $\times 0.3434$ = weight of sulphates as SO_3 in 10 grammes.

$$[\text{BaSO}_4 = 233; \text{SO}_3 = 80; \frac{80}{233} = 0.3434].$$

Estimation of Available CO_2 in Baking-Powder.—An exact method is that recommended by Fresenius in which a small quantity (say 0.5-1 gramme) of the powder is acted upon by water, and the evolved gas absorbed by soda-lime. When all the gas that will come off is absorbed, the remainder of the CO_2 can be evolved by dilute acid and estimated in the same manner; or a fresh sample may be operated on by acid, giving the total CO_2 : this figure minus the available CO_2 gives the unavailable or residual gas.

Estimation of Tartaric Acid.—Wash 5 grammes of the powder into a 500 c.c. flask with 100 c.c. water. Add about 15 c.c. concentrated HCl, and dilute with water up to the mark. When starch and other insoluble matters have settled out, filter the liquid. To 50 c.c. of the filtrate, corresponding with $\frac{1}{2}$ gramme of the powder, add 10 c.c. of a 30 per cent. solution of carbonate of potash, and boil for half an hour. Filter and wash precipitate. Evaporate filtrate and washings to about 10 c.c. Add 4 c.c. glacial acetic acid whilst stirring vigorously, and 100 c.c. 95 per cent. alcohol, and continue the stirring till the precipitate appears crystalline. Stand until precipitate separates out (several hours may be required); decant the liquid through a small filter; wash the precipitate on to the filter with alcohol; wash out the dish with alcohol, and the precipitate with the same, till free from acetic acid. Now boil precipitate, and filter with water in a beaker. Finally, titrate the liquid with decinormal alkali (using phenolphthalein as indicator) to obtain the amount of tartaric acid.

Lead and Arsenic in Tartaric Acid, Citric Acid, and Cream of Tartar.—See Local Government Board Food Reports, No. 2, 1907.

Approximate Atomic Weights :

| | | | | | | | |
|----|----|----|-------|----|----|----|-------|
| Ag | .. | .. | 108.0 | I | .. | .. | 127.0 |
| Al | .. | .. | 27.0 | K | .. | .. | 39.0 |
| As | .. | .. | 75.0 | Mg | .. | .. | 24.0 |
| Ba | .. | .. | 137.0 | Mn | .. | .. | 55.0 |
| Br | .. | .. | 80.0 | N | .. | .. | 14.0 |
| C | .. | .. | 12.0 | Na | .. | .. | 23.0 |
| Ca | .. | .. | 40.0 | O | .. | .. | 16.0 |
| Cl | .. | .. | 35.5 | P | .. | .. | 31.0 |
| Cr | .. | .. | 52.0 | Pb | .. | .. | 206.0 |
| Cu | .. | .. | 63.0 | S | .. | .. | 32.0 |
| Fe | .. | .. | 56.0 | Sn | .. | .. | 119.0 |
| H | .. | .. | 1.0 | Zn | .. | .. | 65.0 |

A litre of water saturated with air at 10° C. dissolves 8.68 c.c. O at N.T.P.

A litre of water saturated with air at 15° C. dissolves 6.96 c.c. O at N.T.P.

A litre of water saturated with air at 20° C. dissolves 6.28 c.c. O at N.T.P.

One hundred grammes of water at 15° C. will dissolve the following amounts expressed in grammes of the salts indicated:

| | | | | | | | |
|---|----|----|--------|-----------------------------------|----|----|---------|
| BaSO ₄ | .. | .. | 0.006 | KBr | .. | .. | 38.500 |
| CaSO ₄ | .. | .. | 0.208 | CaCl ₂ | .. | .. | 40.800 |
| Ba(NO ₃) ₂ | .. | .. | 7.800 | NH ₄ Br | .. | .. | 44.900 |
| NaHCO ₃ | .. | .. | 8.800 | NaBr | .. | .. | 46.500 |
| K ₂ SO ₄ | .. | .. | 9.600 | SrBr ₂ | .. | .. | 50.300 |
| Na ₂ SO ₄ | .. | .. | 11.900 | Mg(NO ₃) ₂ | .. | .. | 50.500 |
| KHCO ₃ | .. | .. | 18.300 | BaBr ₂ | .. | .. | 51.000 |
| KNO ₃ | .. | .. | 21.200 | Ca(NO ₃) ₂ | .. | .. | 53.800 |
| Na ₂ CO ₃ | .. | .. | 22.000 | NH ₄ NO ₃ | .. | .. | 55.300 |
| KCl | .. | .. | 25.000 | KI | .. | .. | 58.500 |
| NH ₄ Cl | .. | .. | 26.500 | NaI | .. | .. | 63.500 |
| (NH ₄) ₂ SO ₄ | .. | .. | 33.200 | BaI ₂ | .. | .. | 66.900 |
| MgSO ₄ | .. | .. | 34.000 | MgCl ₂ | .. | .. | 66.900 |
| NaNO ₃ | .. | .. | 34.200 | CaI ₂ | .. | .. | 67.000 |
| NaCl | .. | .. | 36.100 | K ₂ CO ₃ | .. | .. | 100.000 |

The following salts contain the numbers of molecules of water of crystallization indicated: BaCl₂.2H₂O; Na₂HPO₄.12H₂O; Na₂S₂O₃.5H₂O; Pb(C₂H₃O₂)₂.3H₂O; ZnSO₄.7H₂O; FeSO₄.7H₂O; CuSO₄.5H₂O; AlK(SO₄)₂.12H₂O; MgSO₄.7H₂O; H₂C₂O₄.2H₂O; Cu(NH₄)₂.6H₂O; CuCl₂.2(NH₄)₂Cl₂.2H₂O; NaNH₄HPO₄.4H₂O (micro-cosmic salt); CaCl₂.6H₂O; Na₂SO₄.10H₂O; Na₂B₄O₇.10H₂O; (NH₄)₂(SO₄)₂.6H₂O; MgSO₄.K₂SO₄.6H₂O.

INDEX

- ACARUS domesticus**, 203
 farinæ, 221
Acetyl value, 195
Acid, acetic, 224, 252, 253, 259, 260,
 264, 268
 benzoic, 178
 boracic, 174, 187
 carbolic, 301
 citric, 166, 267, 268, 317
 hypochlorous, 296
 lactic, 252, 253
 malic, 253, 259, 260
 oxalic, 134
 phosphoric, 268
 salicylic, 177, 223
 sulphuric, 224, 267, 268
 sulphurous, 254
 tannic, 257, 258, 259
 tartaric, 259, 267
 value of fat, 192,
Acidity of beer, 253
 of bread, 231
 of milk, 155
 of spirits, 264
 of water, 15, 16, 95
 of wine, 259, 260
Actinomycosis, 233
Adams's process, 157
Adeney's process, 101
Adsorption, 292
Adulteration (see preservatives) of
 beer, 254
 of bread, 231
 of butter, 185, 187, 189
 of cheese, 203, 205
 of cocoa, 285
 of coffee, 281
 of milk, 172
 of mustard, 269
 of pepper, 270
 of sugar, 274
 of tea, 278
 of wines, 259
Æcidium berberidis, 226
Air, 118
 ammonia in, 140, 144, 145
 ammonium sulphide in, 140, 141,
 144, 145
 bacteria in, 146
 bromine in, 141
 carbon dioxide in, 133, 134, 135,
 136
 carbon disulphide in, 141
 carbon monoxide in, 136
 chlorine in, 141
 composition of, 118
 humidity of, 129, 130
 noxious gases in, 144
 oxygen in, 130
 ozone in, 141
 sewer, 143
 sulphur dioxide in, 140
 sulphuretted hydrogen in, 140
 suspended matter in, 143
Albuminoid (organic) ammonia, 41,
 42, 46, 47, 52, 81, 92
Alcohols, 246, 251, 252, 259, 262
 amyl alcohols, 247
 butyl alcohols, 247
 diethyl carbinol, 247
 estimation of alcohol, 252
 ethyl alcohol, 247, 251, 252, 253
 isobutyl carbinol, 247
 methyl alcohol, 246, 247
 butyl carbinol, 247
 propyl alcohols, 247
 table, 265
Alkaline permanganate, 44
Alluvium, 3
Aloes, 254
Alum., 231
Ammonia-free water, 42
Amœba, 69, 70, 75
Anabaena, 10, 72
Anguillulæ, 70, 237
Animal parasites, 236
 spine, 71
Ankylostomum duodenale, 116
Annatto, 179, 199
Antipyrin, 288
Antiseptic, 287
Apjohn's formula, 129
Arrowroot, 220, 221
Arsenic, 254, 317
 estimation of, 255, 256
 in foods, 316
Ascarus lumbricoides, 237, 242
Ascaris, 228

- Ascospores, 228
 Aspergillus glaucus, 203, 222, 223
 Atomic weights, 320
 Azotobacter, 114
- Babcock method, 162
 Bacillus botulinus, 245
 butyricus, 112
 coli communis, 84, 85, 86, 87, 88,
 91, 104, 112, 117, 146, 181,
 245, 289, 293
 denitrificans, 112
 enteritidis sporogenes, 84, 85, 87,
 88, 117, 146, 181, 245
 fluorescens, 200
 fluorescens liquefaciens, 112
 Johne, 182
 Klebs-Löffler, 182
 lactis aerogenes, 112
 mallei, 234
 mesentericus vulgatus, 112
 mist bazillus, 182
 Möller's, 181
 mycoides, 112
 oedematis maligni, 116
 paratyphosus B, 245
 prodigiosus (micrococcus), 5, 89
 proteus vulgaris, 112, 245
 proteus zenkeri, 112
 putrificus, 112
 pyocyaneus, 89
 Rabinowitch, 182
 radicola, 113
 smegma, 182
 subtilis, 112
 supestifer, 245
 tetani, 116
 tuberculosis, 84, 181, 182, 200,
 205, 233, 253
 typhosus, 1, 84, 85, 89, 116, 182,
 289, 293
- Bacteria in air, 146
 in butter, 200
 in meat, 233, 245
 in milk, 180
- Bacterial food-poisoning, 245
 Bacteriological examination of water,
 2, 6
 Bacteriology of water, 83
 Bagshot sands, 3
 Baking-powders, 318
 Barley, 209, 215, 216
 Barometers, 120, 122
 corrections of, 122
 Fortin, 120
 Hooke's, 122
 Kew, 122
- Baudouin's test, 199
 Bean, 211, 219, 221
 Bech.'s test, 199
 Beer, 250
 acidity of, 253
 alcohol in, 252
 aloes in, 254
 arsenic in, 254
 bitters in, 253
 boric acid in, 254
 gentian in, 254
 malt extract in, 253
 salicylic acid in, 254
 sodium chloride in, 254
 sulphurous acid in, 254
- Beggiatoa alba, 10, 71, 73, 74
 Beri-beri, 214
 Bicarbonates, 21, 66
 Birotation ratio, 167
 Bismark brown, 53
 Bitters, 253
 Bleaching of flour, 212
 powder, 296
 Boric acid, 244, 254
 Boulder clay, 3
 Boyle's law, 119
 Brandy, 261, 262
 Bread, 229
 acidity of, 231
 adulteration of, 231
 alum in, 231
 ash of, 230
 composition of, 229
 silica in, 230
- Bromine, 298
 Brownian movement, 305, 306
 Bruchus pisi, 221
 Brucine test, 54
 Bursaria gastris, 10, 73
 Butter, 184
 adulteration of, 185
 bacillus, 182
 bacteria in, 200
 colouring matters in, 199
 composition of, 184
 cottonseed oil in, 199
 curd in, 187
 fat, 187, 189
 acetyl value of, 195
 acid value of, 192
 Hegner value of, 193
 iodine value of, 193
 melting-point of, 190, 194
 microscopic examination of,
 192
 physical properties of, 190
 polarized light test, 198

- Butter fat, Polenske number, 197
 preparation of, 189
 refractive index, 192
 Reichert-Meißl value, 195, 203
 saponification value, 192
 solidification-point, 191
 specific gravity, 190
 titre test, 191
 Valenta's test, 198, 203
 Wijs's test, 194
 preservatives in, 187
 boric acid, 187
 formalin, 187
 nitrates, 187
 salicylates, 187, 188
 sulphites, 187, 189
 saffron in, 200
 salt in, 187
 sesame oil in, 199
 starters in, 200
 turmeric in, 200
 water in, 185
- Cælosphærium, 10, 72
 Caffein, 282, 284
 Calandra granaria, 222
 Calcium, 28
 saccharate, 184
 sulphate, 319
 Cane-sugar, 184, 207, 271
 Carbon (organic), 39
 Carbon dioxide in baking-powders, 319
 in beer, 251
 in water, 65
 Carbonates, 21
 Carchæsius Lachmanni, 73
 Casein, 150
 Catchment area, 2
 Catechu, 280
 Cellulose, 208
 Cereals, 209
 Chalk, 3, 4, 31, 80, 92
 Chamberland bougie, 235
 Champagne, 258
 Chara fragilis, 10, 70
 Charles's law, 119
 Cheese, 201
 adulteration of, 203
 ash, 203
 Brie, 202
 Camembert, 202
 Cheddar, 201, 202
 composition of, 202
 fat in, 203
 foreign fat in, 205
- Cheese, Gorgonzola, 201
 Gruyère, 201
 lactose in, 205
 moulds in, 203
 proteins in, 204
 starch in, 206
 Stilton, 201, 202
 Stracchino, 202
 tubercle bacillus in, 205
 Tyrothrix in, 203
 water in, 203
 water-soluble N, 204
 Chemical analysis of water, 2, 12
 balance, 12
 Chicory, 283
 Chinese silk, 76
 Chloride of lime, 296
 Chlorides in water, 16, 18, 19, 20, 30, 59, 82
 Chlorine, 290, 295, 296, 297, 298
 in air, 141
 Chocolate, 286
 Chromium, 36
 Claret, 257, 258
 Clark's process, 25
 scale, 25
 Claviceps purpurea, 227
 Clostridium pastorianum, 114
 Coal, 3
 Coal-tar, 301
 Coccidia, 239
 Cocoa, 285
 composition of, 285
 Cœnurus cerebialis, 236
 Coffee, 281
 composition of, 281
 Colloidal mercury, 293
 silver, 293
 solutions, 304
 Colour of water, 8
 Colostrum, 166
 Condensed milks, 183
 Conferva bombycina, 73
 Continuous phase, 304
 Copper, 32
 sulphate, 33, 313
 Copper-zinc couple, 55
 Cosmarium, 70
 Cotton fibres, 69, 72, 78
 Cream, 163
 of tartar, 319
 Crenothrix, 8, 10, 71, 73
 Creolins, 289
 Cresols, 301, 308
 Crum's method, 55
 Cryptomonas, 10, 70
 Crystalline rocks, 3

- Cuprous chloride method of estimating CO_2 , 140
Cysticercus bovis, 236
cellulosæ, 236, 240
tenuicollis, 236

Daphnia pulex, 71, 74
 Dangerous water, 11
 Decinormal solutions, 12
Demodex phylloides suis, 236
 Deodorant, 287
 Dew-point, 128, 129
Diamido-benzol, 53
Diastase, 152, 208, 250
Diatoms, 69, 70, 71, 72
Dinitrodiphenylaminesulphoxide, 314
 Diphenylamine test, 54
 Disinfectants, 287
 Disperse phase, 305
Distoma hepaticum, 236, 241
 lanceolatum, 236
 Dotted vessels (chicory), 284
Drepanido tænia lanceolata, 236
Echinococcus multilocularis, 238
 unilocularis, 238
 Egg (*Ascarus lumbricoides*), 69
 (*Tænia solium*), 69
 (*Trichocephalus dispar*), 69
 Elder-leaf, 278
 Emulsions, 305, 306
 Endorina, 72
 Entire flour, 214
 Erosive water, 16
 Esters in spirits, 264
 Ethers in wines, 260
Euplotes charon, 69
Eustrongylus, 237
 Fat (butter), 189
 (milk), 152, 153, 157
 Fault, 4
 Fehling's method, 170
 Pavy modification, 171
Filaria, 237
 Filtrable viruses, 234
 Flax, 77
 Flock, 313
 Flour-improvers, 212, 213
 Formalin, 175, 188, 244, 290, 299, 300, 316
 Frankland's method, 39
 Free and saline ammonia, 41, 42, 46, 47, 52, 81, 92
Friedländer's bacillus, 89
 Fungi, 68
 Furfural, 265
 Fusel oil, 262
 Gases in water, 60
Gentian, 254
 Geology, 3
Gin, 264
 Glaisher's formula, 129
Glenodinium, 10
Glucose, 271, 273
Gluten, 210
Gorgonzola cheese, 201
Graham flour, 213
Greensands, 3, 4
 Griess's method, 53, 55, 57
 Ground water, 5
 curve, 5
Gruyère cheese, 201, 202

Hæmatosporidia, 239
 Hair of insect, 70
 Haldane's apparatus for estimating CO_2 , 315
 method for estimating CO , 137
 Hardness in water, 20, 22, 25
 permanent, 24, 25, 26, 81
 temporary, 24, 25, 26
 total, 24, 26, 81, 92
 Hemp fibre, 69, 77
 Hempel's gas burette, 131
 Hermite solution, 296
 Hock, 258
 Houzeau's test, 142
 Human milk, 149, 153, 154
Humulus lupulus, 251
Humus, 109, 110, 111
Hydra, 69
 Hydrochloric acid in air, 141, 144
Hydrodictyon, 73
 Hydrogen peroxide in air, 142
 as disinfectant, 294, 295
 in milk, 178

 Igneous rocks, 5
 Infant's foods, 205
Infusoria, 69, 70
 Interpretation of chemical analysis
 of water, 79
 Iodoform test, 251
 Ions, 297
 Iron in water, 2, 34, 82
 Ironstones, 3
Isochlors, 17

 Jute, 78

 Kephir, 183
 Kimmeridge clay, 3
 Kjeldahl's method, 98, 165, 184, 314, 315
 Koumis, 183

- Lactalbumin, 151
 Lacteal vessels, 284
 Lactic acid, 155, 253
 Lactoglobulin, 151
 Lactose, 149, 152, 167, 205, 207
 Laplace's formula, 124
 Lard, 205
 Lead in spirits, 262, 317
 in water, 30, 32, 82
 Leffmann-Beam process, 162
 Lemon-juice, 267
 Leptomit^{us} lacteus, 73
 Lias, 3
 Lime-juice, 267
 Limestone, 3
 Linen, 69
 Lolium temulentum, 229
 London clay, 3
 Lunge and Zeckendorf's estimation
 of carbon dioxide, 135

 Magnesium in water, 28
 Maize, 209, 217
 Malt extract, 253
 Manganous chloride, 64
 Marquardt method, 261
 Marsh's test, 255
 Maximum thermometer, 126
 Meat, 253
 inspection, 233
 parasites (animal) in, 236
 preservatives in, 244, 316
 tuberculosis in, 233
 Melosira, 71
 Meridion, 10
 Metallic impurities in water, 261
 Metaphenylene-diamine, 53, 56
 Methyl alcohol, 263
 Methyl butyl carbinol, 247
 Methylene-imino compounds, 244,
 316
 Methyl orange, 25
 Milk, 149
 acidity of, 155
 Adams's process, 157
 adulteration of, 172
 analysis of, 155
 annatto in, 179
 ash, 164
 bacteria in, 181, 182
 benzoic acid, 178
 boracic acid, 174
 casein, 150
 cellular elements of, 183
 citric acid, 166
 colostrum, 166
 colouring matters, 179
 Milk, composition of, 149
 human, 153
 condensed, 183
 cream, 163, 184
 calcium saccharate in, 184
 cane-sugar in, 184
 gelatin in, 184
 starch in, 184
 dried, 183
 fat, 152, 153, 157
 formalin, 175
 heated, 167
 human, 153
 hydrogen peroxide, 178
 lactalbumin, 151
 lactic acid, 155
 lactoglobulin, 151
 lactose, 152, 167, 180
 muco-protein, 151
 mystin, 178
 pasteurized, 167
 reaction of, 155
 Röse-Gottlieb method, 163
 salicylic acid, 177
 sodium carbonate, 178
 solids not fat, 165
 sour, 179
 specific gravity, 155
 streptococci in, 182
 total solids, 163
 turmeric, 179
 Werner-Schmidt method, 160
 Westphal balance, 157
 Millet, 209
 Minimum thermometer, 126
 Moniezia expansa, 236
 Moulds, 203
 Mucor mucedo, 203, 222
 Mucilage cells, 269
 Mustard, 269
 Mustard oil, 269
 Mycoderma aceti, 268
 Myxosporidia, 239

 Navicula, 72
 Nessler's reagent, 42, 57
 New red sandstone, 3
 Nitrates in water, 51, 52, 53, 54, 57,
 82
 Nitric acid in air, 141
 organisms, 39
 Nitrites in water, 51, 52, 53, 82
 Nitrobacter, 112
 Nitrogen as amides, 204
 as ammonia, 205
 as caseoses, 204
 (organic), 39

- Nitrosomonas Europæa, 112
 Nitrous acid in air, 141
 organisms, 39
 Normal solution, 12
 Nostoc, 71

 Oat, 209, 217
 Odour of water, 8
 Enocyanin, 257
 Œstrus bovis, 236
 Old red sandstone, 3
 Oolite, 3
 Ordnance survey, 3
 Organic carbon, 39
 matter in air, 315
 in water, 38
 nitrogen, 39
 Oscillatoria, 72
 Oxidizable organic matter, 47
 Oxidized nitrogen, 51
 Oxygen absorbed from permanganate,
 47, 50, 90, 91, 92, 93, 94, 95,
 96, 97, 99
 dissolved in water, 60, 63, 99,
 100
 Oxyuris vermicularis, 237, 243
 Ozone, 295

 Pandorina, 72
 Paraform, 300
 Paramœcium, 69, 71
 Parasites in meat, 236
 Pasteurized milk, 167
 Pavy-Fehling method, 171
 solution, 275
 Pea, 219
 Peat, 5
 Penicillium glaucum, 203, 222
 Pentastomum tænioides, 239
 Pepper, 269
 Peronospora, 222
 Pettenkofer's method, 133
 Phenol, 289, 292, 302
 Phenolphthalein, 15, 65, 66
 Phenolsulphonic acid, 57
 Phenylhydrazine hydrochloride, 317
 Phenylolids, 303, 304, 306, 307
 Phosphates in water, 27, 28, 30, 82
 Phosphoretted hydrogen, 142
 Phosphorus compounds, 232
 Physical examination of water, 7
 Picric acid, 57
 Piophilæ casei, 203
 Plastering of wine, 259
 Pleurococcus, 70
 Plumbo-solvency, 16, 27, 95
 Poisonous metals in water, 30

 Polarimetry, 168
 Polished rice, 214
 Porosity of soil, 106
 Port wine, 258
 Post-tertiary deposits, 3
 Potassium sulphate in wine, 260
 permanganate, 298
 Potato, 220
 Pouchet's aeroscope, 143
 Primary deposits, 3
 Proteus vulgaris, 89
 Puccinia graminis, 225
 Purbeck marble, 3
 Putrefaction, 39

 Qualitative examination of air, 144
 Quassia, 254

 Rain, 6
 Reaction of water, 13, 91, 92, 93
 Reinsch's test, 256
 Relative humidity, 130
 Resin acids, 307
 Rice, 209
 Rideal-Walker coefficient, 312
 method, 304, 307
 Rivularia, 10
 Röse's method, 263
 Rum, 264
 Rye, 209

 Saccharomyces ellipsoideus, 257
 Salicylic acid, 224
 Sarcosporidia, 239
 Sea-water, 95
 Self-registering thermometer, 125
 Sesame oil, 199
 Sewage, 1, 2, 81, 92, 94, 96
 effluents, 60
 fungus, 9
 Shales, 3
 Shallow wells, 3
 Sherry, 258
 Silica, 29, 230
 Silk, 76
 Six's thermometer, 125
 Sloe-leaf, 278
 Soaps, 20, 21
 Sodium chloride in beer, 254
 tetrathionate, 49, 60, 62
 thiosulphate, 49, 60, 63
 Soil, 105
 bacteria in, 111
 clay, 107
 humus, 109
 lime, 108
 magnesia, 108